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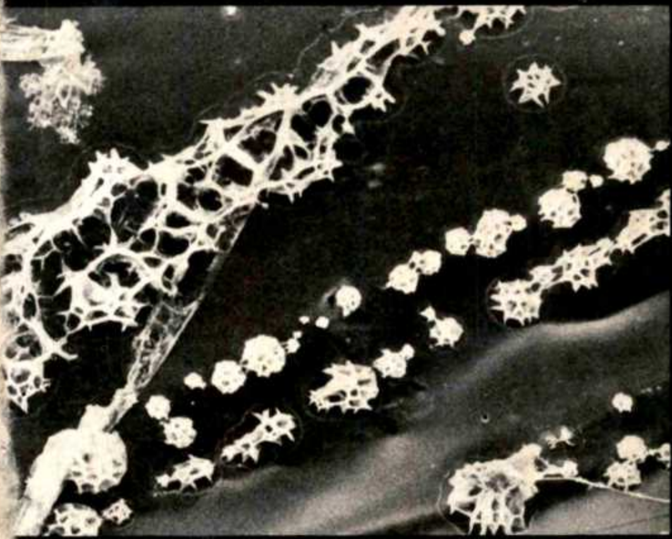

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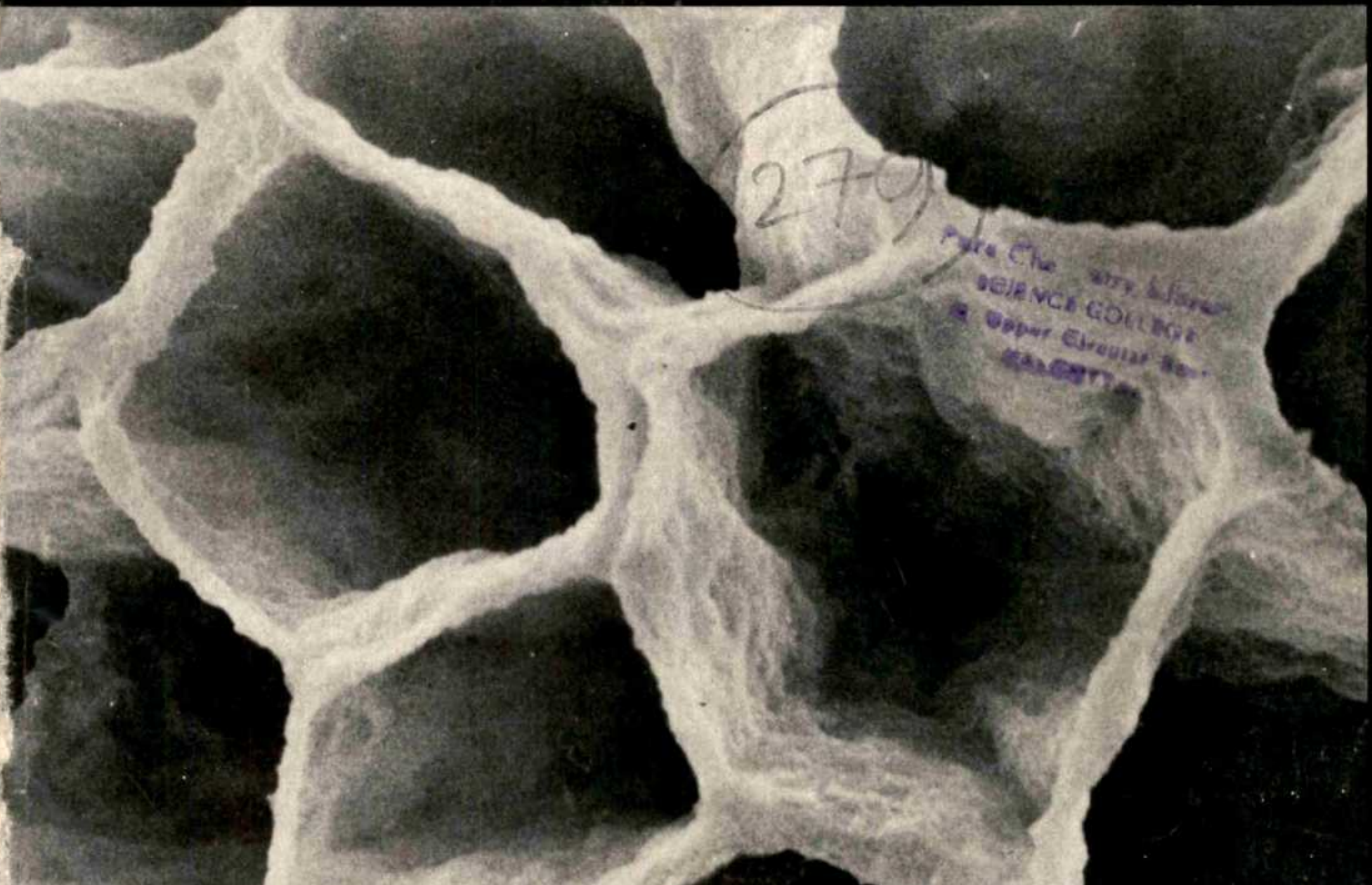

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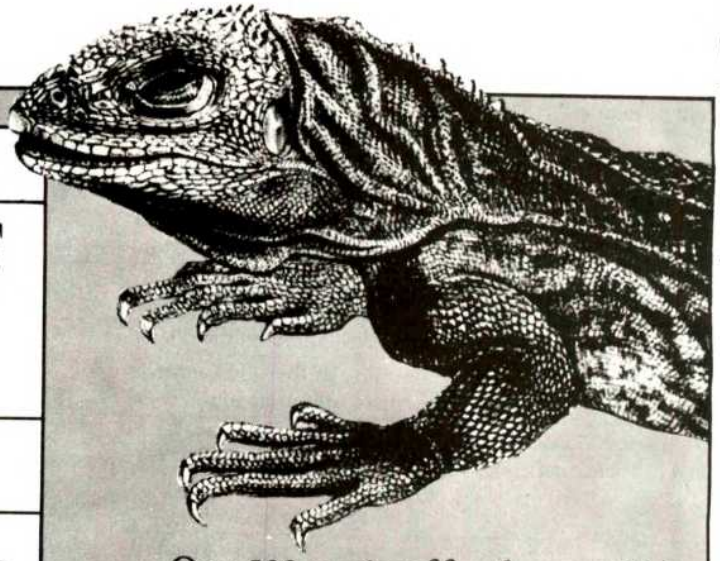

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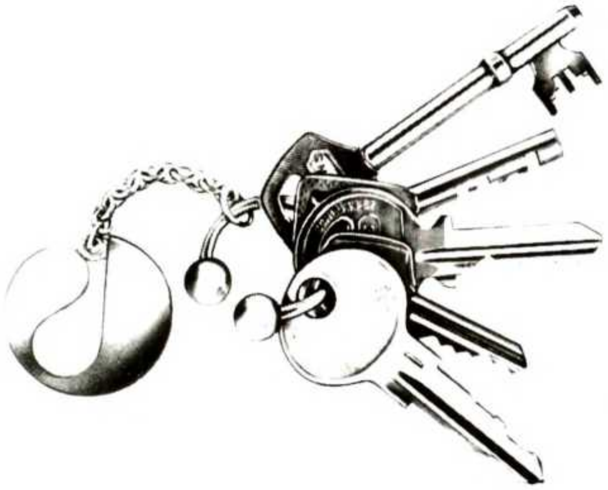

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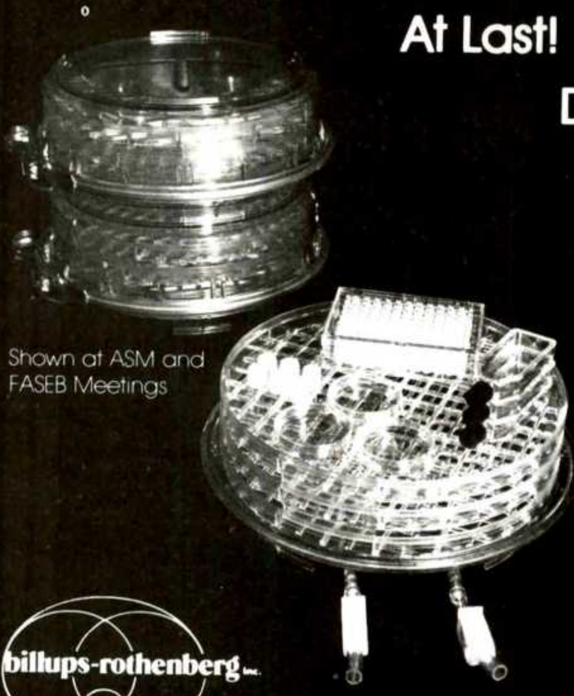

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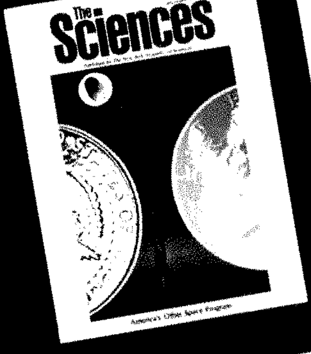

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2. O JANNE, K KONTULA and R VIHKO, *Journal of Steroid Biochemistry*, vol. 7, pp 1061 1068 (1976)
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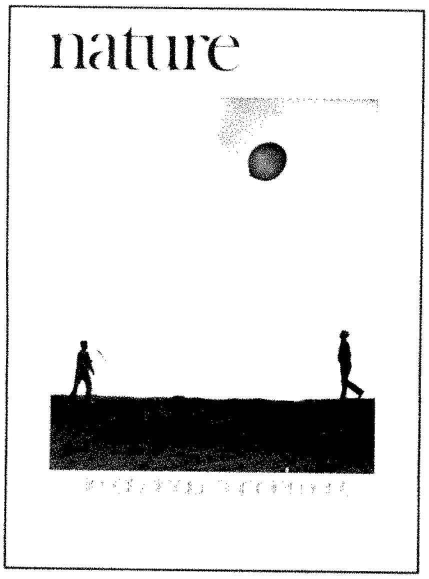

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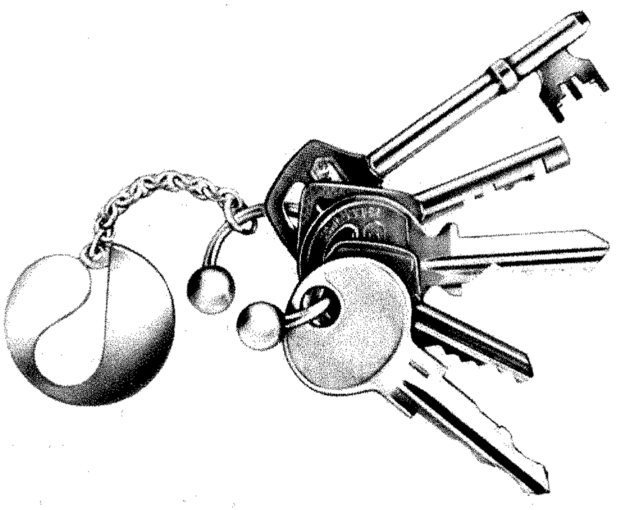

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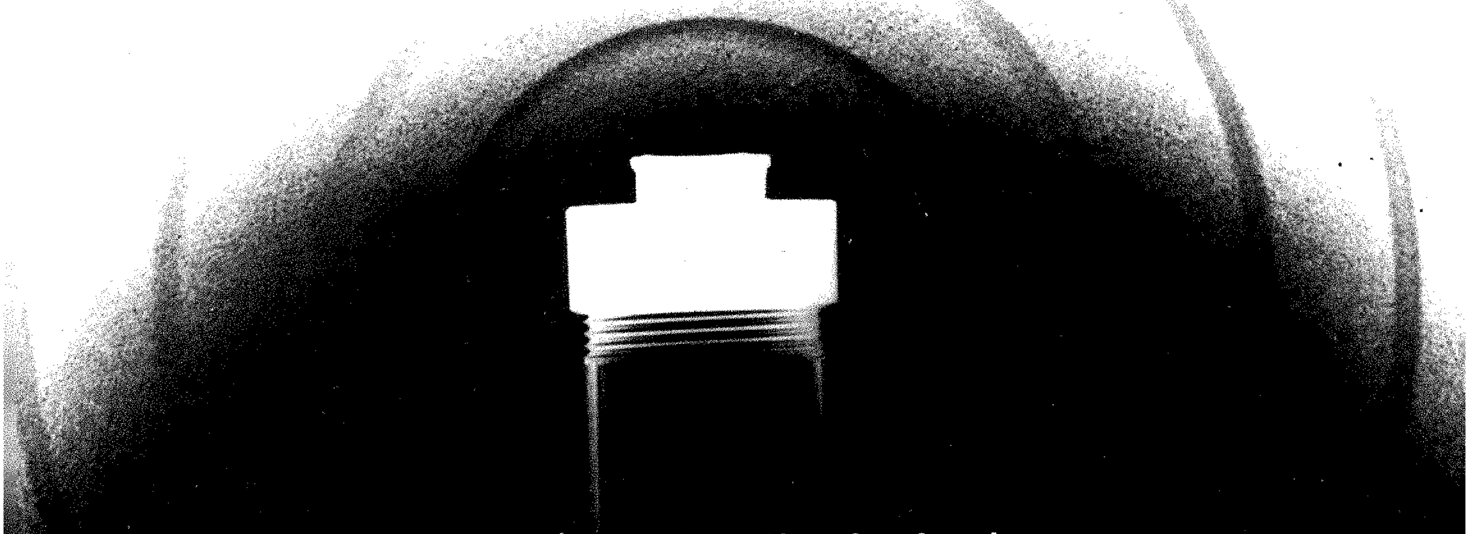

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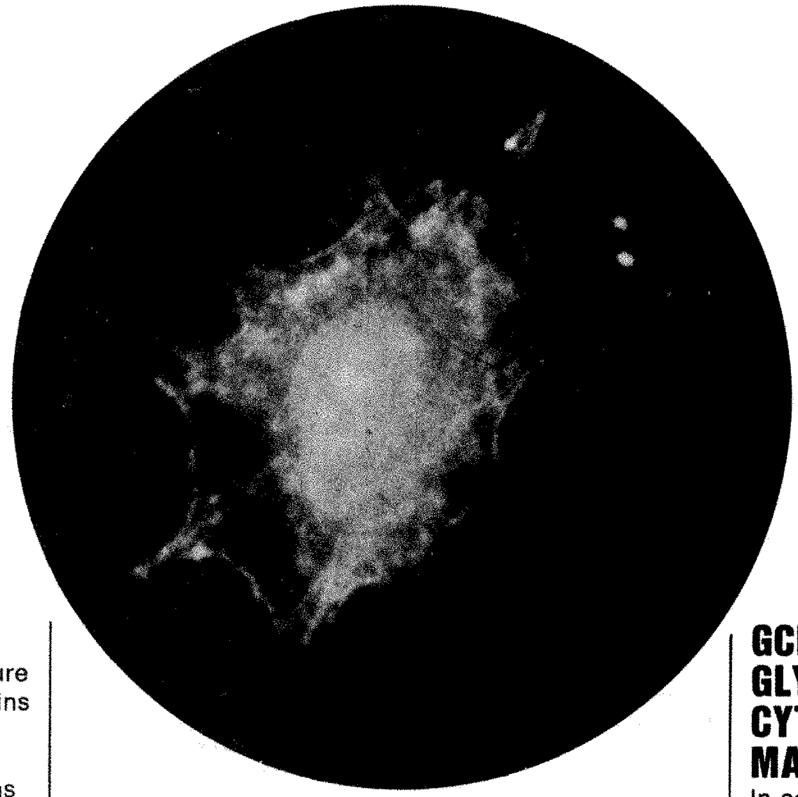

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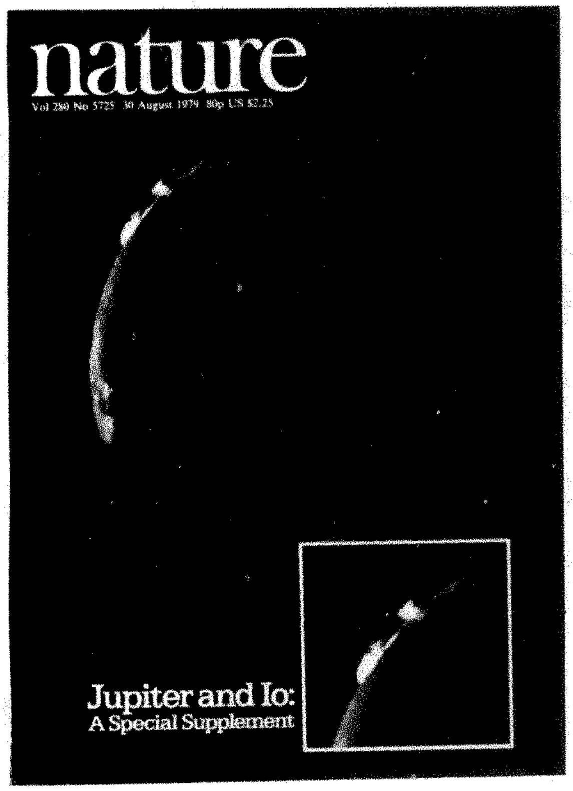

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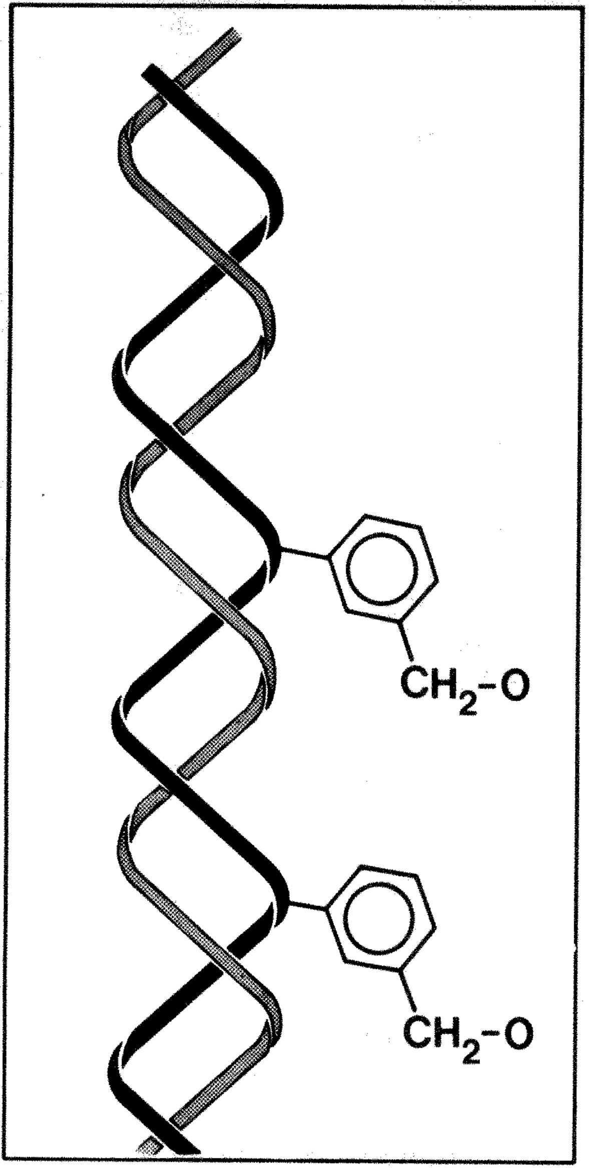

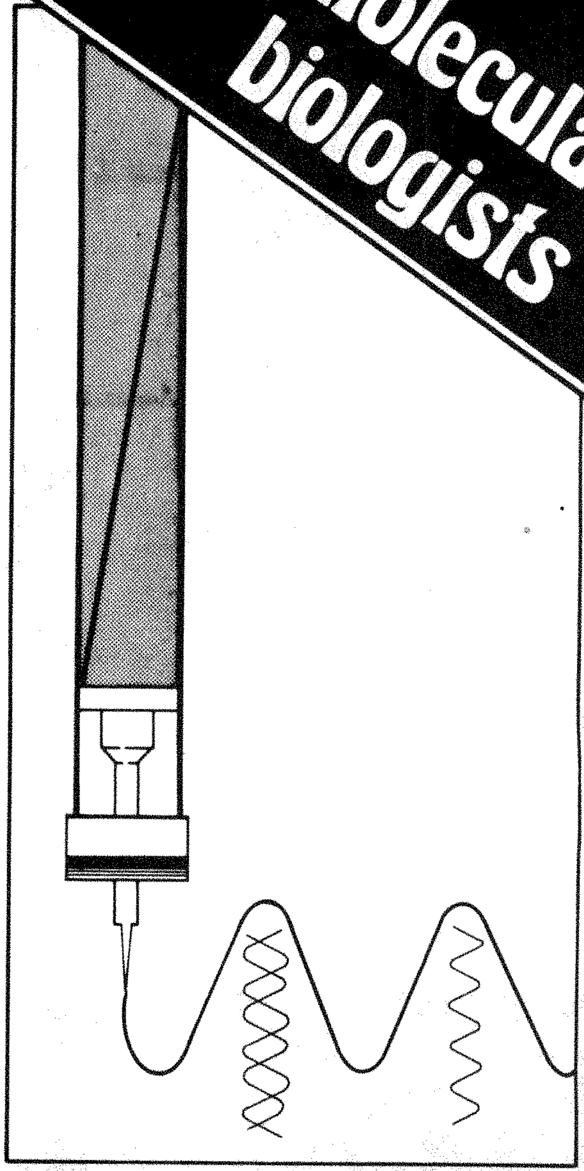

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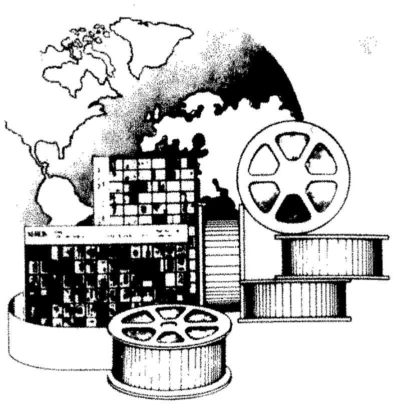

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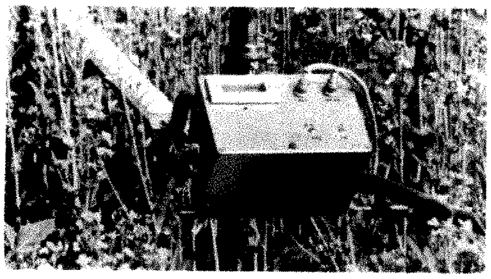

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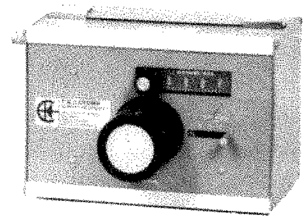

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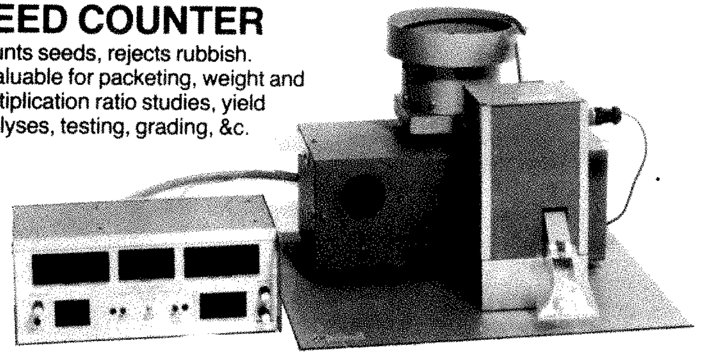

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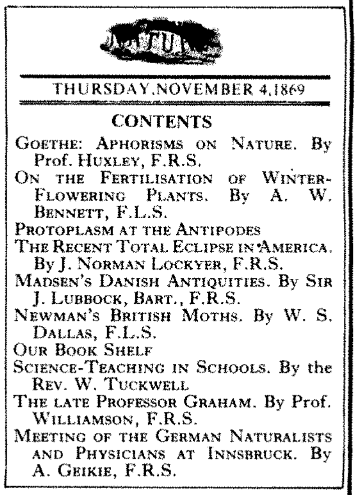

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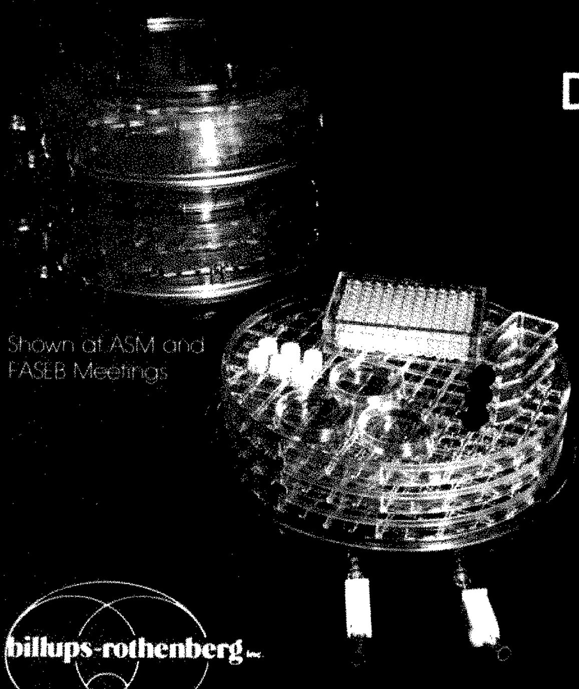

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Nuclear power: the critics must be heard

WHEN announcing the UK government's decision to build at least one nuclear reactor per year during the decade beginning 1982 (see page 3) Mr David Howell, Energy Secretary, placed great emphasis on the need for safety.

After the accident at the Three Mile Island nuclear power plant in Pennsylvania, US last March, public fears about the safety of nuclear power are justifiably strong. And in the UK, that fear is likely to be heightened by the government's probable intention to base the new programme on the pressurised water reactor — of a similar type to that at Three Mile Island — rather than the UK developed advanced gas cooled reactor.

Mr Howell was therefore right to stress the need for safety and to reaffirm the government's intention of holding a public inquiry on the safety of the PWR before the first is built. This view is endorsed by the new House of Commons Select Committee on Energy which recently announced that the PWR is to be its first topic for study. It is to call on Mr Howell and representatives from the nuclear industry and the nuclear opponents as witnesses and is to pay special attention to safety.

However, what will be most important over the next two years leading up to the public inquiry and indeed, throughout the subsequent construction of any power stations, is that Mr Howell's promise is seen publicly to be adherred to. The need for the nuclear industry to be entirely open about its plans is greater now than it has ever been. This is not the time for it to hide behind the government's decision in the hope that it will be out of the limelight. More than ever before, public attention will be turned on it and the public will expect its opinions to be taken into account.

The major lesson which must be learned from previous nuclear debates in many countries is the need for the critics of nuclear power to have complete access to information. We endorse the government's decision to release unabridged safety reports on all nuclear power stations but would go one step further. We recommend that the Nuclear Installations Inspectorate consider the opinions of the serious nuclear critics during any future safety assessments of nuclear power stations. This means that the critics must have the same rights of access to design and safety documents and the same rights to make site inspections as do NII inspectors.

Greater openness will not be to the detriment of the nuclear industry. On the contrary, an industry which is seen publicly to be taking the right decisions about safety is one which will receive support at home and orders from abroad. Where safety is concerned, the industry should not worry about the need for commercial secrecy because the industry which is the most successful in demonstrating the safety of its reactors will be the one most successful in selling them.

Finally, the current decision should not automatically lead to the first step along the road to a major commercial fast breeder reactor programme. Rather it should be seen as a way of keeping our options open well into the 1990s. In the meantime, research — and particularly the development — of alternative sources of energy and new uses for coal should be stepped up. Only after equivalent funding will it be possible to make a balanced judgement between — say — fast breeders and wave power. ☐

David Davies' editorship ends

FOR six and a half years *Nature*, and much of the world's scientific community, has enjoyed Dr David Davies' work as its editor. A man of character, he has impressed his personality and leadership on the journal. A firm believer, as I am, that the specifically human characteristic of adaptability requires change to express itself, to lead to the maximum contribution and real job satisfaction, he has always lived by his precept.

Once again, he felt that the time had come to move on to a radically different activity. He has become director of the newly established northern centre for the Dartington Trust, at Barnstaple, Devon. Much as we will all miss him from his editorial desk, his new task of nurturing vitality and amenities in the rural parts of North Devon will certainly give full scope to his drive and creative thinking.

He came to *Nature* from work as a geophysicist in MIT's Lincoln Laboratory in the US, with particular concentration on the intricate field of the seismic discrimination and the detectability of underground nuclear explosions — efforts continually central to the much hoped for complete test ban treaty. He had come to geophysics from physics at Cambridge University.

He threw himself into his new tasks at *Nature* with characteristic flair and drive. His international outlook, the result of much travel and experience of work in two countries, was translated into efforts which matured into a remarkable success of making *Nature* a journal concerned with the progress, health, organisation and vitality of science in all countries of the world. We should perhaps be particularly grateful for the coverage of countries of the developing world, but his own intimate knowledge of the United States scene, his selection of correspondents in Canada and on the European continent, his ensuring the reporting of what goes on in the USSR and Eastern Europe, all this has lead to a global outlook of educational benefit to all *Nature's* readers.

Personally, I have most enjoyed his editorials, pithy, hard-hitting and relevant. One may not have agreed with every one of them, but none was dull. But the journal's success is above all based on the original science it contains. It was no mean effort to have kept and increased its attraction to scientists everywhere for the rapid but responsible publication of relevant new advances. To have added to this central core of original letters a series of important and novel review-type articles in every issue has materially added to the value of the journal. The book reviews too have been a source of much enjoyment and profit to me.

His six and a half years with *Nature* have made an impact on all its readers. We all wish him well in his new and very different tasks.

Hermann Bondi

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United States

NAS calls for global aerosol ban

An international ban on non-essential uses of chlorofluorocarbon (CFC) aerosol propellants is now the most effective way to reduce the dangers to health and food supplies from further depletion of the ozone layer — says a report published by the National Academy of Sciences. **David Dickson** reports.

THE US banned aerosol spray cans a year ago, and this has led to a 50% drop in the use of CFCs.

The report points out that there remain many other uses of CFCs — for example in automobile air conditioning units, or in the production of flexible plastic foam cushioning — equally in need of control. Such non-propellant emissions could bring the total CFC emissions back up to previous levels within seven to ten years.

Reducing such uses, however, is likely to be difficult, since in many cases there are no acceptable substitutes. But the report points out that progress could be achieved if other industrialised nations would agree to a ban on non-essential aerosols: "should all countries decide to take action comparable with that already taken by the United States and Sweden, between one third and one half of the world's present CFC released would be avoided.

"Such a reduction would be a great step forward in decreasing the threat to the world's food supply, even though the magnitude of this threat is uncertain, and its advantages would far outweigh the relatively small costs of substituting alternative propellants and devices."

The report was prepared by the Committee on Impacts of Stratospheric Change and the Committee on Alternatives for the Reduction of Chlorofluorocarbon Emissions of the Academy's National Research Council. It was commissioned by the US Environmental Protection Agency.

Its strong message adds further fuel to the controversies that have been building up in other industrialised countries since the hypothesis that CFC emissions could seriously deplete the ozone layer — subsequently increasing the exposure of humans, animals and plant-life to harmful ultra-violet radiation — was first raised by US scientists in 1974.

Two months ago, another academy committee confirmed earlier conclusions that, if emissions continue at the present rate, the amount of ozone in the atmosphere is likely ultimately to be depleted by about 16%, half taking place within the next 30 years.

A few countries, in particular Sweden and Norway (expected soon to be joined by Canada) have agreed to follow the US example. But West Germany, France, the UK, Japan and Italy (who, together with the US, account for 90% of the West's production of CFCs) have refused to impose stringent controls.

There are signs that their position may be shifting. Member countries of the European Economic Community, for example, which earlier this year agreed voluntarily to reduce non-essential aerosol usage by 30% by 1982, last week agreed to advance the date by six months, and to hold back on additional investment in industrial uses of CFCs.

The US however, would like things to move much faster. The report recommends the complete elimination of non-essential aerosols on a global basis. Dr John W Tukey, of Bell Laboratories, chairman of one of the panels, said last week: "The US has done it, and it wasn't very painful."

The new report examines in detail some of the consequences of the depletion in the ozone layer predicted by the companion study. And it discusses some of the alternative technologies, such as sealed air conditioning units, that might reduce or replace CFC-emitting processes.

The most obvious human health effect, it says, would be a further increase in skin cancer, (melanoma), already one of the most rapidly increasing forms of cancer in the US. The report predicts several thousand more cases of melanoma a year, "of which a substantial fraction would be fatal."

But the committee expresses greatest concern over the effect on global food supplies. "Here we know enough to be concerned, but not enough to predict what the biological effects would be," says Dr Tukey.

As DNA is affected by exposure to solar ultra-violet radiation the effect on the growth and reproduction of animals and plants would be expected to lower the yields of farms and fisheries.

Experiments indicate that some crops, such as sugar beets, tomatoes and corn, might be "significantly affected" by a 16% ozone depletion.

The report points to the particular threat to aquatic food systems studies of the UV sensitivity of young anchovies indicate that this commercially-important food source now lives near the limit of its solar-UV tolerance when at the surface of the water, as do certain crab and shrimp larva. "A 16% reduction in ozone concentration would be distinctly harmful to them in a surface location," it says.

Discussing the non-aerosol uses of CFCS, the report says that in many cases alternative technologies already exist, in particular for the agents used in blowing flexible pastic foam, and for the solvents used for cleaning metal or for sterilising medical supplies.

In other cases, however, controlling the use of CFCS is not likely to be so simple. It picks out their use in automobile air conditioning systems, the larges single sources of CFCS remaining in the US.

The prospects of reducing emissions from this source are "not good", though modifications to minimise leakage could yield significant reductions without burdensome costs.

The report outlines a variety of measures to restrict non-aerosol uses, from a complete ban on certain applications — the simplest approach, but "not always advantageous" — to a judicious mixture of tax measures, quotas on specific uses, and public edcuation.

The ozone problem differs from earlier environmental threats, since wherever CFCs are emitted, the ultimate effect is global. It is therefore the forerunner of another serious problem: the increased atmospheric concentration of carbon dioxide. It concludes: "the nations of the world collectively need to devise mechanisms for dealing with problems of this sort, assessing the scientific evidence as it develops and finding ways to agree on effective control actions, since these are not problems that can be adequately dealt with by the actions of only a few nations." □

Heavy water reactors are less proliferation resistant

Although making more efficient use of uranium, heavy water reactors are less proliferation resistant than either high temperature gas-cooled reactors or light-water reactors, according to the draft report of the US Department of Energy's Non-Proliferation Alternative Systems Assessment Programme. The report, published last week in Washington for public comment, suggests that AGRs might have an application in the industrial process-heat market. In general, however, it suggests that the US can minimise the risks of proliferation by pushing ahead with the development of LWRs, and with liquid-metal fast breeder reactors. "In order to influence the liquid-metal fast breeder reactor programs of other countries, not only must the US be a member of the fast-breeder development community, but it also needs to exercise leadership in developing the technological measures which would reduce the poliferation risk of fast breeder reactors," the report says.

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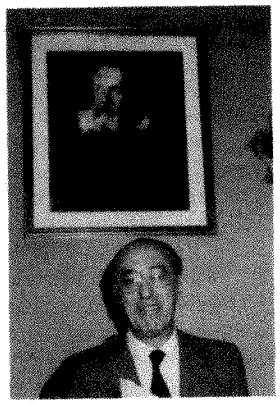
Italy

Confronting the Euro agencies

Vito Scalia, Italy's science minister, alerted *Nature* shortly before Christmas (see 20 December p 768) to the three-pronged attack he was to make on European research organisations. Speaking in the room in the Rome convent where Galileo was tried (right) Scalia demanded that Italy get fairer treatment from ESA, that CERN delay its decision on a new Director-General, and that the EEC adopt an experiment on nuclear reactor meltdown. The results of his efforts are now becoming plain, as **Robert Walgate** reports.



ITALY'S representatives at CERN, the European organisation for subnuclear research, withdrew from the 19 December Council meeting and refused to endorse the appointment of German Professor Herwig Schopper, as new Director-General from 1 January 1981.

The Italians are thus carrying out their threat to "consider seriously dissociating" Italy from CERN. One of the present joint CERN Directors-General, Dr John Adams, offered to visit Rome to mediate in the dispute — which is principally between Italy and Germany — but Rome refused.

The delegation is apparently still smarting from the rebuff it received — at a preliminary meeting — to its proposal that the future programmes of CERN should be "better clarified" before an appointment is made. Italy fears that Germany is not altogether sincere in its commitment to CERN's future, as it is considering a proposal for an accelerator (HERA) that Italy believes is competitive with CERN's LEP (see page 5).

The Italian government has its own candidate for the CERN Director-Generalship, Professor Antonino Zichichi. But Italian scientists working at CERN were nervous of their delegation's tactics, as they rely on CERN and other non-Italian laboratories for their livelihood.

JET under threat

THE European Commission's Super Sara experiment on nuclear reactor meltdown is now supported by eight of the nine community members, with only France objecting.

At a meeting in Brussels of energy ministers of the nine, Italy — the strongest supporter of Super Sara — swung a number of other objectors (including Britain) behind her. But Pierre Aigrain, France's Minister of Science, continued to hold strong objections.

France claims that its similar experiment at Cadarache is more advanced than Super Sara — which would be conducted at the EEC Joint Research Centre at Ispra, north Italy, on an isolated circuit within the reactor ESSOR.

France's objections have led Italy to act on its threat to hold up once again the EEC's 1980-85 budget for energy research.

ESA absorbs objections

ITALY'S attack on European research organisations succeeded in one respect: a tacit agreement of the Council of the European Space Agency to adjust the budget in Italy's favour to the tune of 27 million units of account (mua) (about $40 million).

Italy had argued that it had lost some 32 mua over the allocation of contracts to build Spacelab, the orbiting laboratory which will be flown by the Space Shuttle.

The adjustments should be confirmed at a meeting on 23 January. They will come in the form of a reduced contribution over four years, and a greater allocation of Spacelab follow-on projects. ☐

United Kingdom

Government plumps for PWR power station

THE UK government has decided to embark on a major new nuclear programme. At least one nuclear power station is to be built each year during the decade beginning 1982 and the nuclear industry is to be revitalised to cope with the demands to be made of it. The estimated cost of the programme — to be paid for by the Central Electricity Generating Board — is £10-12 billion at 1979 prices. It is expected to increase the nuclear contribution to electricity supply from 12% to over 30% by the early 1990s.

Mr David howell, Secretary of State for Energy, said when announcing the decision that the UK needed a nuclear programme of this size if it was to maintain the type of society it has now into the 1990s, when North Seal oil and gas production would decrease. The size of the programme, equivalent to about 15,000 MW, was based on Department of Energy forecasts of energy demand, assuming a 20% saving in consumption from conservation, he said.

A substantial programme was also needed to build up and maintain a strong industry; the lack of orders over the past decade had led to its decline. "If we are to reverse this trend and ensure that the industry is on a sound footing, we must act now", said Mr Howell. Under the reorganisation planned for the industry, the National Nuclear Corporation (NNC) and the Nuclear Power Company are to be brought under the umbrella of one board. The new NNC will then have total responsibility for management of all nuclear projects.

One of its first tasks will be the design of a pressurised water reactor under license from Westinghouse. In line with the previous Labour Government's decision, the Conservatives intend that the first power station of the programme will be a PWR. The design must be approved for safety by the Nuclear Installations Inspectorate and must be subject to a public inquiry. The NNC estimates that it could be ready for this by the end of 1981, and that construction could begin in 1982.

The NNC is also to assume responsibility for managing the UK's advanced gas cooled reactor (AGR) programme. By building two more AGRs as well as one PWR, the CEGB hopes that the final choice of reactor can be left for some time. However Mr Howell has said that a commitment to one system or the other would have to be made fairly early on in the programme.

The major advantage of the PWR over the AGR is capital cost. The CEGB estimates that the capital cost per MW is £750,000 for an AGR and £680,000 for a PWR.

● The British nuclear industry and the NII have released preliminary statements on the implications of the accident at Three Mile Island. They conclude that the recommendations of the President's commission, set up to investigate the accident, do not apply to the UK. Fault was found with the regulatory procedure in the US and the standard of operator training but not with the basic PWR design. Most of the suggestions for improvement, they say, are already incorporated in the UK regulatory procedure.

Judy Redfearn

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Brezhnev counts on Academy of Sciences to organise applied research

LAST month, the Soviet Academy of Sciences met to discuss the role of scientists in solving the urgent problems facing the national economy. This was on instructions from the Party Central Committee and the government, and was a response to Mr Brezhnev's November address to the Central Committee. In his swingeing attack on Soviet planning and production, Brezhnev singled out for castigation a number of ministries (including railways, power and ferrous metallurgy), the State Planning Committee and the "bureaucratic and local interests" which "weaken the strength of the plan". In tackling the "vital tasks", both of production and planning, said Brezhnev, the Party counts on the active assistance of scientists, the USSR Academy of Sciences, the Republic and branch academies and all scientific research institutes. "The State Committee for Science and Technology", he added, "must also work in a more energetic manner".

This is not the first time that Brezhnev has called on the academy for assistance. At the Twenty-Fifth Party Congress in 1976, he had declared that the academy was to act as cheif coordinator of all scientific work in the country. This caused some confusion, not least to the academicians themselves. It now seems certain, however, that what Brezhev meant was that the academy should coordinate fundamental research throughout the USSR, stressing the need to implement the practical applications of such research.

At the same time, a number of specialists from the academy were involved (together with officials of the State Planning Committee, the relevant ministries and the State Committee for Science and Technology) in working out a "complex programme" for the development of science and technology up to 1990. Work on this programme began in 1972, under the general direction of a special commission of the academy presidium, but by summer 1979 it was still not completed. In the course of work on the "complex programme", there have been numerous set-backs; perhaps the greatest being the change from forecasts for individual technologies with little coordination between them (some 150 in the first drafts) to 25 "integrated" programmes, 17 for science and technology and eight for social questions, covering broader fields such as energy, health, industrial materials, and demographic trends.

How far existing plans for the "complex programme" will be affected by the latest appeal to the academy is not clear. Addressing the assembly, the President of the academy, Anatolii P. Aleksandrov stressed the Soviet Union's continuous shortfall of ferrous metals, cement and fertiliser, the need to pay greater attention to the "economic utilisation" of energy, raw materials and agricultural produce.

Nikolai Baibakov, Chairman of the State Planning Committee and a Deputy Chairman of the USSR Council of Ministers, stressed the need for a long-term comprehensive programme for transport, including pipelines for pulp, pipelines using containers, magnetic suspension transport and linear motor trains. The automation of manual labour (a major problem for the Soviet Union with its falling demographic trend) was also stressed. Baibakov commended the academy's current research on energy-saving types of equipment and new types of polymer, which, he said, should ultimately save the economy some 1000 million roubles.

Media comments on the session, presumably from official releases, stressed the need for a new comprehensive plan for science and technology up to the year 2000, giving priority to new regions in the far north, Siberia, and the Soviet Far East.

Setting the tone for such developments, Baibakov noted that "socialist planning has always been 'target-orientated'", citing Lenin's plan to electrify the whole country, the construction of heavy industry in the 1930s, the Virgins lands scheme of the early 1960s, and the current construction of the Baikal-Amur Mainline railway.

His remarks have some suggestion that science and technology might take on the status of a "crash programme". (This would not be out of key with the economic "mini-reform" proposed last July). Aleksandrov, the President of the Academy has, incidentally, considerable experience of this type of priority development (nuclear research) as had his predecessor Mstyslav Keldysh (space). Such crash programmes have been particularly successful in sectors with some defence connections (since defence falls outside the main planning structure). Any attempt to introduce wide-scale crash planning would, however, appear to be counter-productive — there would be too many priorities competing for too few resources.

The main weakness of Soviet science planning, however, remains the point at which production takes over from research. Since the mid 1960s the planners have set considerable store on closer links between research and academic institutes on the one hand, and local farms or factories on the other. The classic case of such cooperation is the Siberian branch of the academy at Novosibirsk. Here a network of research institutes, design centres and experimental production factories has been set up. These are maintained by, and officially subordinate to, the relevant ministries, but the various institutes of the Academy of Sciences are responsible for the scientific leadership.

Recently, a number of *ad hoc* "science centres" were established throughout the Soviet Union, often simply by formally linking existing research and industrial establishments. Many of these centres, Baibakov told the academy, are "observed to be dissipating their resources" and wasting time on "second grade problems", which are not "in the mainstream of technical progress". As a result, "many ministries (presumably those censured by Brezhnev) have been unable to fulfil their plans for science and technology".

Since the Siberian branch of the academy was established, a number of academics, including its chairman academician G I Marchuk, have pointed out that the dual control system could lead to precisely this kind of dissipation. However, this has always been presented as a danger. By concentrating too much on the minutiae of day-to-day problems, the ministry could distract researchers and resources from basic and far-reaching tasks. Academician B Paton of the Ukrainian Academy of Sciences would prefer that even the design and pilot-production facilities should be subordinate to the academy, so as to obviate this hazard.

Baibakov's remarks, however, seem intended to exonerate the ministry planners and, perhaps, by implication, shift a greater responsibility to the academics. The relationship between the Academy of Sciences (traditionally orientated to fundamental research) and the state committee for Science and Technology, with its stress on production is always a delicate one. Although Brezhnev has censured the state committee and called once again on the academy's assistance, the state committee has recently taken over a duty which would normally be considered the prerogative of the academy. At the forthcoming Hamburg "scientific forum", where most of the Helsinki signatories will be represented by their academies of science, the Soviet delegation will be headed by the Chairman of the State Committee.

Vera Rich

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NEWS IN BRIEF

CERN discloses plans for new accelerator

LEP, a particle accelerator to collide 22-130 GeV electrons with their antiparticles, positrons, at the same energy, would begin construction near Geneva in early 1982 if plans announced at last week's CERN Council meeting are accepted. John Adams, Director-General of the Centre for european nuclear physics (CERN), said that he planned to submit a firm proposal for LEP to the Council — the supreme body of CERN on which all member states are represented — in June 1980. A decision could be taken in June 1981.

LEP would probably be 5 km in radius, and be built underground. It could be built without increasing the present CERN budget of 593 million Sw Fr per year, and no new staff would be needed, said Adams; but the intersecting storage rings (for proton-proton collisions) would be phased out, and the old 8 GeV synchrocyclotron — CERN's first machine — reduced to serve only the low energy physics 'Isotope separator on line' (ISOLDE), which is widely used by Scandinavian members of CERN and has produced much useful data on short-lived isotopes.

USSR to license use of advanced energy technologies

THE Soviet Union will license the use of 30 processes for eliminating the high sulphur content of coal and for developing electricity production through magnetohydrodynamics to the US. The agreement signed by the USSR foreign trade organisation Licensintorg and the US firm Control Data Corporation, is a consequence of a deal made several years ago when Control Data supplied a superconducting magnet to the USSR for use in MHD experiments. In return, Control Data saved the need to build a MHD test plant of its own.

US to stop funding labour-saving agriculture research

THE US Secretary of Agriculture Mr Bob Bergland, has announced that federal research funds distributed to land-grant colleges and universities can no longer be used to support research into the mechanisation of agricultural processes leading to reduced employment for farm workers.

Such research has come under increasing criticism in recent years for contributing to unemployment among farmworkers, particularly among migrant workers who had previously been dependent on seasonal agricultural work for a major proportion of their income. Critics charge that the federal research funds which are distributed to land-grant colleges on a pro-rata basis under the terms of the Hatch Act are merely serving to enrich large food-producing corporations.

However, Secretary Bergland's move was criticised by Dr Charles E Hess, head of the College of Agricultural and Environmental Science at the University of California, Davis, who said that "to suggest halting research which significantly contributes to enhancing agricultural productivity and efficiency is irrational and irresponsible."

France and China sign agreement on geological research

AFTER eight days of meetings and visits to Chinese laboratories, Pierre Aigrain, the French Secretary of State for Research, signed an agreement on 14 December with Deng Xiao-ping, the Chinese vice-Premier, to provide for Franco-Chinese cooperation in geological research. The agreement gives the go-ahead to three French missions to study the north slope of the Himalayas in 1981, 1982, and 1983. In return France will provide laboratory equipment for Chinese geological research. Other areas of possible cooperation are teacher exchanges, the evaluation by French specialists of Chinese mineral reserves, particularly chromium, and the establishment of a Chinese geological information bank. Mr Deng Xiao-ping called the agreement a step in the direction of correcting the "loss of scientific talent due to the Cultural Revolution".

Ariane makes it to the top

ARIANE, Europe's — but mostly France's — very own space launcher, went aloft on Christmas Eve (24 December) only 2 km away from its planned trajectory. A few days after the launch, measurements showed that the payload — a small test satellite — was in an orbit with perigee (closest approach to Earth) of 202.6 km, apogee (furthest distance) 36,019 km, with an inclination of the plane of the orbit to the equator of 17°.

The launch appeared to demonstrate that the hitches which caused the abandonment of two launch attempts within a week were indeed minor. They are attributed to small instrumentation malfunctions.

The greatest achievement — and surprise — of the launch was the perfect operation of the hydrogen-burning third stage, which had not yet passed its ground qualification tests.

European Space Agency press announcements before the launch had hedged around what would be considered a 'successful' launch, urging journalists to count mere ignition of the third stage a 'success'. There will be three more trials in 1980; one more 'success' will be considered a 'qualification' of Ariane for its first commercial launches in 1981.

GMAG invites proposals for scale-up

THE UK Genetic Manipulation Advisory Group has issued guidelines for work with recombinant DNA "involving volumes of 10 litres or more". All proposals for "scale up" will be judged on the basis of a site visit as such will be considered case by case rather than by a set of strict rules. GMAG will examine the biological aspects of proposals for "suitable levels of disablement and containment".

Physical plant will also have to be examined for pressure safety, leaks or cracking and safety and emergency procedures will be inspected as will sterilisation provisions for waste products. A GMAG official said that the requirements are not beyond industry's present procedures for biological manufacture as for example in vaccine and antibiotic productions. "We want to encourage not discourage applications. Industrial procedures are usually much safer than laboratory work and if the proposals are consistent with safety we would be quite happy".

Saccharin confirmed as weak human carcinogen

STUDIES carried out by the US National Cancer Institute have shown that heavy users of artificial sweeteners, especially those who consume diet drinks and other sugar substitutes, have a 60% increased risk of bladder cancer.

The study was based on interviews with more than 3000 bladder cancer patients and 6000 healthy people. It revealed "no increased risk" in the overall population being studied, but that those who used six or more servings a day of the sugar substitute saccharin, or two or more eight-ounce diet drinks, increased the risk of bladder cancer by 60%.

Following announcement of the results of the study, the Food and Drug Administration, whose attempt to ban the use of saccharin has been blocked by Congress, announced that it confirmed its previous contentions that artificial sweeteners posed a cancer risk to humans. Dr Jere E Goyan, Commissioner of the FDA, said that the study was consistent which previous animal studies and scientist's conclusions that saccharin is a "weak carcinogen", in particular acting in conjunction with other causes of cancer, such as cigarette smoking.

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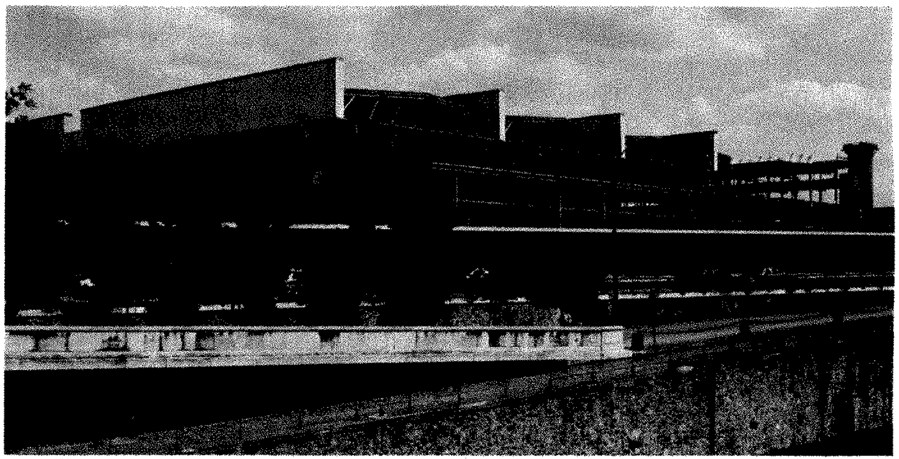
FEATURES

France plans new science museum

FRANCE is soon to publish a report proposing the construction of the largest science museum in the world. Compiled on the instructions of the President Giscard d'Estaing by Professor Maurice Lévy and a committee of 11, the report was completed last October after a 12-month study. However, Giscard d'Estaing has still not publicly released it; clearly there is a lively debate going on within the government about just how large a commitment France can afford to make to such a project at a time of economic squeeze. But there is no doubt that the project will go ahead in some form; even if money is hard to find, the advantages are too large to ignore.



La Villette: a possible home for the new science museum.

First, there is the location. A disused abattoir called La Villette squats in the north-east corner of Paris. Its grounds cover 52 hectares, making it the largest open space in Paris outside the Bois de Boulogne. Abandoned almost ten years ago, the prey of property speculation and grandiose schemes, La Villette has long been the biggest property scandal in Paris. Thus its planned transformation into a park and a new Museum of Sciences and Industry (with a concert auditorium to be added later) will remove a painful reminder of a rather disreputable past. The conversion could be ready in five years at an estimated cost of 363 million FF.

Second, there is the equally scandalous condition of Paris's present science museum, the Palace of Discovery. Housed in a crumbling building constructed in the 19th century as a "temporary" exhibition hall, much of the equipment dates from the 1930s when Jean Perrin created it. Conceived of as one of a series of museums, it had to crowd in examples of all the sciences when the plans for the others were abandoned. Perrin's idea of "familiarising our visitors with the fundamental research by which science is created, through daily re-creations of the great experiments" has, through lack of funds and support, largely degenerated into formal lecture-demonstrations by underpaid staff to audiences increasingly made up of reluctant schoolchildren and middle-aged professionals. Meanwhile, the Conservatory of Arts and Crafts, set up at the time of the Revolution to be a collection of all the "originals of instruments or machines invented or perfected" in France, has remained a hodgepodge of machines, with almost no representation of very old, or very recent technologies, or those not originating in France.

So the decision to build a new museum of science, in La Villette, has a compelling logic.

Professor Lévy sees the museum as a counterweight to the way science is taught and popularised in France. As in most countries, the public view science as a formalised, completed structure, rigidly demarcated as the domain of "experts", pursuing their unfathomable goals by unknowable means. In France this is particularly marked, and leads to a virtual absence of public debate on scientific and technological questions. The new museum, Lévy feels, must be the initiator of such debates. "Science", he points out, "is too important to be left to the scientists."

Hitherto, most museums have followed one of three patterns. One such is typified by the Palace of Discovery: the hierarchial initiation of the layperson into the mysteries of science by the "expert", the quasi-academic lecture and demonstration. The conservatory of Arts and Crafts is an example of the second type: a dusty collection of artefacts, the frozen detritus of past accomplishments. Finally, there is the third kind, found in most postwar museums, where there is a tendency to represent science as a series of disjointed tricks, black boxes which, when their buttons are pushed by the visitor, dutifuly trot out their magical effects.

But now modern technology and the increasingly sophisticated demands of the public, Lévy claims, has made possible "not an amelioration but a mutation" in the concept of a museum. New methods of communication will allow people to participate in scientific and technological decisions by giving them real access to the basic conceptual foundations of science and their practical consequences through active participation and debate.

This will happen in two ways. First, the flexibility of modern techniques permits "branching", the creation of an autonomous set of subthemes so that each museum visitor will be able to follow his or her own interests and needs in a certain subject, from basic concept to detailed application. Second, the museum at La Villette will make use of the "science in the streets" movement where scientists at conferences also present their ideas in local public forums, answering questions and stimulating debate. La Villette plans to take this up in the form of a series of weekend events held every two or three months in which scientists and technologists would engage the public at open meetings at the museum as equal partners in a discussion of the consequences of what they have done.

There are a number of questions however which have been raised about the project. Bertrand Gille, an eminent French historian of technology, has pointed out that the relations of science to technology is far from unproblematic. A lack of historical perspective, the propagation of the idea that there is "one" technology flowing effortlessly from scientific advance are "received ideas" which still inform much thinking about the new museum, and tend to undermine the possibility of the critical approach that the museum is supposed encourage. Furthermore, Michel Biezunski, one of the originators of the interactive computer systems forming the base of the museum, is concerned about the control of the content of the displays. The public depends on the information in the exhibitions, and since both science and industry have a vested interest in stressing the positive side, where is the space for informed, critical debate?

Answering these criticisms, Lévy insists that the museum will try to show science as a "human endeavour", possibly by the inclusion of examples of scientific failures as well as successes. Further, he also insists that the museum staff, not government or industry, control the content of all exhibitions. But doubts persist. It is, after all, in the very nature of a museum to present historical successes, for they have become "knowledge". It may be felt that few people would come to see displays of technical failure, even though such exhibits might really illuminate how science works.

Jim Ritter

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UK underestimates cost of Trident deterrent

Britain has decided to renew its independent nuclear deterrent, probably by buying the submarine-launched Trident C-4 missile from the United States. **Judith Reppy**, Visiting Fellow at the Science Policy Research Unit at the University of Sussex, argues that this would be a questionable decision on economic grounds

AT present the British deterrent force consists of a fleet of four submarines armed with Polaris A-3 missiles that were supplied by the United States on terms well below cost in the 1960s, a largesse not likely to be repeated. The timing of the decision on renewing the deterrent is forced by the expected deterioration of the Polaris submarine hulls after 25 years of service, that is, by about 1993. Given the lead time needed for development and production of a new submarine, a decision is necessary soon. Postponement might mean abandoning the submarine-based deterrent, although choice of a different technology, such as air-launched cruise missiles, would imply a different timetable.

Coincidentally, NATO has just decided to push ahead with the modernisation of its 'theatre' nuclear weapons by deploying US Pershing II and Ground Launched Cruise Missiles (GLCMs); 160 of the latter will be based in England. Although in one sense these theatre missiles could be regarded as 'strategic' weapons — since like Polaris they will be able to reach targets within the Soviet Union — from an operational and financial perspective they are quite different. A new independent British deterrent force will be paid for by Britain, and, although it will be integrated into NATO forces, Britain will reserve the right to fire the missiles on its own. The theatre nuclear missiles will be assigned to NATO, but will be paid for by the United States and be under US control.

The decision to replace the Polaris fleet is first and foremost a political decision, based implicitly (for there has been no public discussion) on a certain view of Britain's status in the world and its relations with the US and its other NATO allies. The decision also has economic and technological consequences.

How much will a new submarine-based nuclear deterrent cost the British taxpayer? The operating costs of the Polaris fleet are not particularly large — only £126 million out of a total defence budget of £8,558 million in 1979 — but the investment in the design and production of a new submarine and missile will be heavy. Ian Smart in his 1977 Chatham House paper 'The Future of the British Nuclear Deterrent' estimated the cost of a replacement programme at £2,245-2,925 million at 1976 prices. That figure, swollen by inflation, stands now at perhaps £5,000 million for five submarines and their nuclear missiles.

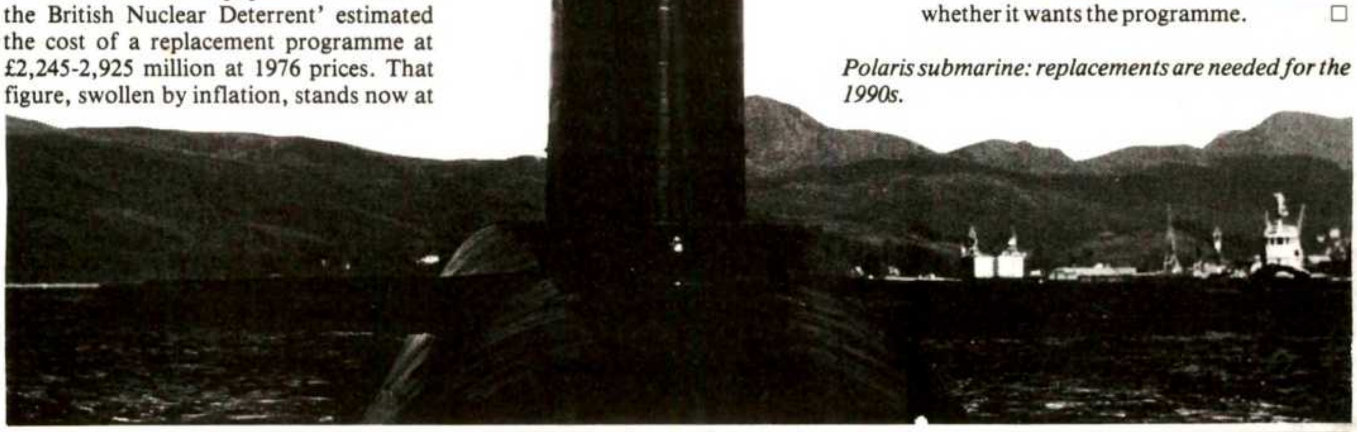
There is reason, however, to question whether even this sum will pay the costs of replacing the Polaris fleet. The United States expects to spend over $23,000 million (about £10,500 million) on a fleet of thirteen new submarines, and missiles; 62% of this is attributable to the cost of the submarine (see 'Trident — the US Experience: ADIU Report, Vol. 1 No 4, December 1979). The US Trident submarine is a huge boat, 560 feet long, and it has proved very expensive to build, costing over $1600 million (£730 million) per boat. But even though Britain would surely choose a more modest design, it is still likely that it would cost more than Smart's estimate. That estimate allocated only 40% of the total programme costs to the submarine, or roughly £240 million per boat (1976 prices). The US figure suggests that is too low.

British shipbuilding capacity has shrunk since the Polaris submarines were constructed in the 1960s, and only the Vickers Barrow yard now builds nuclear submarines. To build the new submarines at Barrow would take up capacity needed for other Navy programmes, whereas investing in new capacity elsewhere would entail costs not included in the Smart estimate. It can be argued that to invest in additional specialised industrial capacity to build five units of a product for which there is no other market would be economic folly.

Beyond these budgetary costs lie important opportunity costs, both with respect to other categories of public spending, such as education and health, and within the defence category itself. The share of the budget required for submarine design and construction will almost certainly pinch other elements of defence spending. Considering the emphasis placed on shortages of conventional weapons in the government's assessment of the military balance, it is perhaps surprising that tanks or air defence weapons are not considered a more urgent priority.

Given the large sums of money involved, the Trident decision also represents an important element in government funding for new technology. It is likely that the government will purchase the missiles from the United States, but develop and produce the warheads in Britain as was done for Polaris. Expertise in the technology of multiple independently targeted re-entry vehicles (MIRVs) will be acquired from the US. These capabilities, however, are not likely to spread into the civilian economy; they are too specialised for direct transfer, and the military market itself is so narrow that further internally-generated technological growth is not probable. The expenditure of more than £1,000 million on R & D for the Trident missile must be justified solely in military terms.

But the most disturbing aspect of the government's decision has been the absence of any public debate on the validity of the arguments for renewing the independent deterrent and the advantages and drawbacks of the various technological options. The public will pay the costs; it deserves a chance to consider whether it wants the programme. ☐



Polaris submarine: replacements are needed for the 1990s.

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Science in the 80s: the need for political involvement

As Secretary of State for Education and Science in the last Labour Government, Mrs Shirley Williams (below) took an above average interest in the health of the scientific community. **David Dickson** talked recently to her at Harvard University, where she has just been a visiting fellow

DD: Ten years ago you wrote about the "party being over" for scientists. How do you feel that statement has worked out?

SW: Throughout the 1970s it has been more difficult for scientists to get money; to that extent my prediction was broadly right. In particular, the gradual reduction in per capita spending on the universities has meant that the British research councils have had to find a greater proportion of the total research expenditure than was the case in the golden years of the late 1950s and early 1960s. But I hadn't at the time thought that not only would the party be over, but that those who went to the party would be spending their time cleaning up afterwards — which seems to be the situation now. I hoped that we could get back to a steady, albeit modest, rate of growth, at least for the research councils; it's now clear that we will not see what I was hoping would be about a 3-4% real growth rate per annum over four to five years.

DD: What has been the main impact of economic stringencies in political terms?

SW: So far, the science budget has not tended to be very involved in either the politics of being cut or being expanded. It tends to stand slightly outside the rest of the political process, and therefore to run along with whatever is the overall tendency. In a period of expansion, the science budget tends to grow alongside; similarly it tends to contract during a period of contraction. And one of the things I was very anxious to do — and just succeeded in doing towards the end of my time as Secretary of State — was to cut the linkage between seeing the science budget as, in a sense, merely another expression of the "science and education" budget. I thought it had to be treated separately, because it had quite different cases to be made for it. I don't believe, for example, that the science budget is appropriately linked to the massive impact of demographic trends, on the education budget, whether rising as in the late 60s or now declining.

DD: What type of arguments did you use in Cabinet a year ago to obtain support for increased funding for research?

SW: I tried to explain in ways that would be understandable to the non-scientist — and to some extent I used myself as a litmus paper — what was going on in science, and what the prospects were for reasonable breakthroughs in some fields, or at least for some very fruitful work. Examples might be the work on laser technology being carried out by the Science Research Council, or the Medical Research Council's work in fields such as senile dementia and schizophrenia, research which offered some prospect of making substantial savings in the health service in the future, if one could find ways of treating people outside mental hospitals.

The idea was not necessarily to say "this is what the work being done is about" but rather "these are examples which readily lend themselves to being thought about in terms of their impact on other people's programmes". I didn't feel there had been a sufficient attempt to explain the research council's activities, as distinct from saying "here's the science budget; I want a 2% growth for it". In other words, there had not really been an argument about the priorities for scientific research as against the priorities for other things, such as defence or social security.

My own belief is that, although scientists don't like to think that research is involved in the political process, it has to be, at least to the extent that science is explained as proselytised about. It wasn't really necessary at the time of the great Robbins expansion of higher education in the 1960s, because the dual support system bore the expansion on its back. Now that system is threatened by demographic shifts, as well as the general cutting back of the higher education budget — which makes it important for scientists and the research councils to learn to put across what they are doing, and build up a lobby of support.

DD: Turning to the social aspects of scientific growth, how do you feel the relationship between the scientific community and the political community has changed in the 70s?

SW: In the narrow sense of the two communities, I would firstly sum it up by saying that the scientific community resisted getting involved, or even communicating much, with the political community, and it certainly seemed to me that such communication was very important. The process under which, for example, the science and technology committee of the House of Commons has tended to cross-question civil servants, and even to some extent the heads of research councils — even though the witnesses sometimes feel they are not revealing vast knowledge — will only work effectively if scientists are willing to make themselves available to the political community. I would criticise scientists in Britain by saying that, in general, they have not been all that readily available, and have been a bit suspicious of people taking time off from the lab to talk to "those amateurs out there". I think that's rather a mistake.

DD: You have frequently suggested that there should be more of a scientific lobby. Do you feel there has been any movement in this direction?

SW: A little. The research councils, and particularly the heads of the research councils, have done much more to come out and explain what they are doing. And insofar as the barriers between the sciences are beginning to break down — for example through joint research projects between the different councils — as well as to some extent the barriers between the scientists and the press, then the research councils are pushing in the right direction.

DD: But once money begins to get tight, are you not required to make a better case justifying support?.

SW: Yes, and I think that's right. One parallel that has always struck me forcefully is that you could increase the arts budget by 15%, and the entire arts lobby would shout its head off about how mean it was, because they always want at least 25% increase a year. But if you increase the science budget by 5% in real terms — say 11 or 12% in money terms — scientists would always be rather grateful. Now I think that the arts lobby overdid it, but they have a large lobby of people who will write letters to the newspapers, speak in the House of Lords, and so on. The scientists — who, thank goodness, are at long last beginning to appear in the Houses of Parliament — are not so ready, don't react so quickly, don't fight so hard. I wouldn't want to push it so far as the arts lobby does, which may even be slightly counterproductive, but I still think there is something to be said for closing the gap a bit.

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DD: How do you feel about the way that concerns about the social impacts of science have been institutionalised — the Genetic Manipulation Advisory Group is the obvious example — and of the way that the scientific community has responded.

SW: There have been many types of responses. GMAG is only one example, and indeed the fact that the Association of Scientific, Technical and Managerial Staffs recently held a conference on GMAG is a good example of how questions about the social impacts of science have been spread beyond the small community of scientists and administrators that directly interweave with them. The Windscale Inquiry is another good example, which was rather well handled, if one compares it with some of the tremendous clashes that there have been in France and Germany. The whole idea of having an inquiry of that kind, with a judge in charge, but passing the conclusions on to the House of Commons for a free vote, was rather an innovative way of going about an area where science and social policy come up against one another.

One example where this went badly was in the area of dangerous pathogens — the smallpox case at the Univeristy of Birmingham; and that was because the dangerous pathogens' rules and regulations pre-date public interest in the field of science and social policy, so that what we had was a relatively secretive and relatively unpublicised system of control and regulation. Immediately something went wrong, the Shooter Report on the smallpox case was able to pinpoint all sorts of holes in the system, because it hadn't really been looked at. People had become complacent about it; it wasn't within the light of the public domain. GMAG was; nuclear power to some extent is, although I would like to see a broader range of public interest brought in; and this is also particularly true in the area of advanced medical science.

As for GMAG, as a device for meeting public concern and, in the initial stages, for allowing experiments to be conducted in closely controlled situations, it had a great deal to be said for it. As a step into a whole new area of science with a great number of public fears associated with it, you only needed to have one accident for the whole of genetic engineering to be at great risk, and for some parliamentarians to say "ban the lot". Indeed there was quite a strong move in Parliament to say that we should not do some of the experiments that fell into the containment categories. And so although I know now that some scientists, and even more industrialists, would criticise GMAG as being exceptionally restrictive, nevertheless as a way of showing that public fears were being taken seriously it was an important beginning. The risks may be low; but what's at stake is so high that the risk-benefit equation does involve a great deal.

What bothers me about the US is that the controls and regulations, such as they have been — I use that tense advisedly — do not really extend very much into the private sector. And, of course, there are countries, such as Switzerland, where the private sector is relatively unregulated. Given that what we are looking at should be an international system of regulation — you can erect national systems, but what difference does that make to a virus? — one has to make some sort of a compromise. I realise that it would almost certainly not be as rigorous as the current GMAG rules to be widely acceptable, but one hopes it would have rather more sensible safeguards, especially with regards to category III and category IV experiments. Britain might have a more regulated national system than some other countries; but the general principles of good practice at the bottom end and pretty strict regulation at the top end is something we ought to try to get across through the European Science Foundation, and in other ways.

DD: The US is currently experiencing a political reaction against regulation, and is caught in the trap while move is being learnt about potential environmental and health hazards, the less regulation becomes politically acceptable. How do you correlate social and political needs?

SW: The way I see it, the US often needs regulation to help make sense of its legislation, which has often been cast in the form that will generate sufficient support — legislation emerging from a process of creating a coalition to get it through. Regulation then becomes the way that you rationalise this rather strange cross-path of legislation. If one takes the fields of education or employment — or for that matter safety and health — one finds that the regulations are extremely detailed and precise, but that they are often changed and amended in the light of events. So that you find people responsible for regulation almost going round the bend with the sheer weight, change and detail of the regulation. And that, in turn, leads to the kind of antithesis of people like Senator Edward Kennedy, for all that he is a liberal senator, moving against regulation.

DD: How do you see the future prospects for science in Britain? What are the new political pressures likely to be in the 1980s?

SW: One important need is for the leaders of the scientific community to get across the idea of lead-times; in other words, to try to get agreement as far as possible that a substantial chunk of the budget ought, as far as possible, to be insulated from year-to-year changes. I'd like to see the budget split between extra money that could be used quickly, and money which requires to be committed for some time, since one of the most difficult things to do is to set up a team of people and then to discover a year or two later that the team is chopped off from its funds, or from half of its funds, just when it is getting going.

Secondly it is important to move in the direction that Geoffrey Allen of the SRC talks about, namely providing fellowships on a four- or five-year basis for young scientists. I am very concerned that the profile of the academic world is such that unless we are very careful, some of the most able of the new generation of young scientists will not get jobs in universities.

DD: But looking at the question the other way round, what new challenges can you see science having to face from the political body — or from the social body — to which it will have to respond?

SW: One area in which a great deal of thought is needed is the extent to which the 'sophistication factor' is likely to have an impact on certain major areas of public finance, particularly in health. A scientist once said to me that if heart transplants had been generally successful, the National Health Service would be in even more trouble than it is, and that seems to me correct. We should be worrying whether the sophistication factor, particularly in medicine, will in practice mean that one is budgeting against the needs of the great majority of the population. It is extremely important to notice how anti-distributive the effects of advanced medical technology can be. Look at the US where medicine has become so expensive because of the technology that, in practice reasonably decent intermediate technology medicine is out of the range of part of the population.

I believe that the government — particularly people concerned with the health field, though pensions and mental health also come into this — should decide, together with scientists, what profile of research is likely to ease the position of hard-pressed health budgets, and what research is likely to make it worse.

People should be aware of the costs as well as the benefits of successful research. Even in the US, in the discussions that I have followed here on medical insurance schemes, almost nobody has taken up the theme of the massive consequences of rapidly advancing medical technology in terms of health care. If I can be blunt, I think that in ten years time it will be impossible to finance any effective public medical service in the US, because a great range of less expensive but reasonably effective technologies will have been ruled out.

DD: Does this mean you see the science budget more and more closely linked to other areas of government spending and social policy? How can the social impact issues be brought into the policy process?

SW: I think that it has to be done at every level. Certainly the research councils should take such matters into consideration. But one would also have to have a kind of parallel system, parallel meetings of interest groups in the House of Commons and in the mass media. This would mean the scientific community being brought together with economists and social scientists more often to look at the implications of certain advances. ☐

CORRESPONDENCE

Indian science fails to meet basic needs of the country

Sɪʀ, — Anil Agarwal's article on 'Indian Science' brought out several interesting points. However, he has failed to raise some basic issues. For example, while India is spending money for highly sophisticated technology such as space and nuclear research, science and technology, even in their crudest form, have not reached the mass of Indian villagers, for whom hunger and starvation are an everyday reality. Indian science policy was not able to prevent the Bihar famine and recent floods and cyclone disasters. It has, however, led to the creation of bureaucratic research institutions and to the mushroom growth of CSIR research laboratories. It is, in fact, only the continuation of a policy which existed in colonial times. Research institutions such as CSIR, the Atomic Energy Commission and the Defence Research Laboratories are prestige institutions. Most Government bodies are bureaucratic institutions controlled by a few individuals.

India has built up a sizeable infrastructure for research and industry, but technology for manufacturing common household goods, such as dry batteries or condensed milk, still has to be imported. Indian scientists have given no thought to the need of basic technology in their country. Despite the green revolution, agricultural wages have declined or stagnated in real terms. The population of cities is increasing annually by about 5% because of the influx of the impoverished from the countryside.

The present attempts to formulate a science policy do not account well enough for education. The type of science education given in Indian universities is imported from western countries, and has no relevance to Indian society. A large amount of money has been spent on universities, research institutions and engineering colleges. At the moment there are more than 3,000,000 students in universities, more than one third of them being science students. By the end of 1979 the figure will rise to more than 5,000,000.

Indian universities have become factories for producing graduates. But why expand like this when there are not enough jobs for those who already have degrees? Recently there were more than 40,000 applicants for 40 vacancies; and for a few vacancies in nationalised banks there were so many applications that they had to be collected in vanloads. Unemployment among graduates runs at more than 30% and in large cities the situation is really alarming. Even though most universities provide undergraduate science and technology courses, the standard is pretty bad. That of science teaching in private colleges (which are affiliated to the universities but run on a commercial basis) is far below normal. Most teachers do not have sufficient qualifications to teach at this level and laboratory facilities are very bad.

What is the point of producing graduates who do not come up to reasonable standards and who do not serve any useful purpose in Indian society? Instead, science education should aim to teach basic ideas on a *mass scale*. The standard of science teaching in schools must be improved before talking about undergraduate study. Most schools in small towns have to be content with spirit lamps, and experiments are not devised to make maximum use of existing facilities.

It would, for example, be a good idea to use experimental kits (like those devised by the Open University) which could be stored in small towns, each store forming the nucleus of a science centre. Mobile service units, manned by trained teachers and operating from those centres, could go to villages and explain to children, with the help of simple experiments, the concepts of basic science and technology. So far Indian science education has no relevance to Indian society.

Although a lot of noise has been made in favour of appropriate technology, it has failed to make any impact on rural development in India. This is because appropriate technology by definition is an indigeneous self-generating process arising as a response to the economic and social needs of various forms and levels of society instigated and initiated by the users of existing technology. Such technologies will not work satisfactorily until farmers have experienced the benefits of communal production and projects and are thus able to design and operate such equipment. Unless the desire for change and for an appreciably higher standard of living takes place in the peasant communities (which is happening among Punjab and Hariyana farmers) new techniques will not be accepted or exploited fully.

At present the keystone of Indian science education is elitism and personal achievement rather than social responsibility; centralisation rather than dispersion; urban rather than rural development; consumption rather than aesthetic development. What is important and essential is that basic science and technology should be oriented towards the needs of the Indian society. Above all, the masses must be carried with technological development and not isolated from it.

Yours faithfully,

A. Vᴀɪᴅʏᴀɴᴀᴛʜᴀɴ

The Open University, Milton Keynes, UK

Nuclear secrets

Sɪʀ,—We concur with the sentiment expressed in your editorial *Nuclear secrets: no clear frontiers* (18 October page 511) that the "narrower question of American security should yield to the broader one of global non-proliferation". But we hope you did not mean that articles about hydrogen bombs should not be published simply because of "sincerely held views" that such articles contribute to proliferation.

Neither of the disputed documents could be of aid to a state in the initial acquisition of nuclear weapons, because the documents discuss thermonuclear weapons, for which a fission weapon capability is a prerequisite. Moreover, most of the dialogue and court proceedings *was* in the broader context of global non-proliferation.

Months ago, before the temporary restraining order was issued by Judge Warren, we technically reviewed Morland's article for *The Progressive*. The article and letter do not contain design details; rather they discuss various — oftentimes incorrectly applied — concepts already known to scientists throughout the world. It is therefore difficult to understand how such an article can further affect the already precarious international situation we now face.

Technical information is essential to informed political debate on an enormous range of issues. For example, thinking only of current policy debate concerning nuclear weapons and nuclear arms limitation, we might list the following: the SALT treaty, the necessity of the MX missile, its siting alternatives, and a comprehensive nuclear test ban.

The US and other nuclear weapons-states must face the fact that five nations, without help, have successfully undertaken the industrial and scientific effort necessary to make hydrogen bombs. The average length of time to achieve a fusion explosion has been five years after first testing a fission device. That this average was as long as five years is more likely due to political indecision and industrial development time rather than a lack of understanding of elementary concepts.

Motivation and resources are far more important determinants than underlying concepts. It is still prudent for governments to avoid authenticating (through their powers of classification) partially correct public information, such as the Morland article and various letters. But mainly they should concentrate on protecting their own ultrasensitive official documents containing weapons-design data, for example UCRL-4725 and -5280, which the US Government erred in making available to the public.

The issues involved in the case go far beyond the question of weapons data alone. For example, the US Government in its brief to the 7th Circuit Court of Appeals in Chicago raised the argument that "technical data" are not constitutionally protected under the First Amendment because they are not an "essential part of any exposition of ideas" and are not of any "social value as a step to truth". In a world as technologically oriented as ours, such an argument is not only nonsensical and false, it is dangerous to the functioning of free societies.

One of the issues raised by Morland is that unnecessary secrecy can tranquillize public debate. Partly because of reliance on technology-denial and secrecy, several US Administrations have avoided a moratorium on testing, production, or deployment of nuclear weapons — although such restraint would retard proliferation far more substantially than does the illusion of security through technology-denial and secrecy. The most urgent need is a universal nuclear test ban, which might be introduced as an amendment to the Nuclear Non-Proliferation Treaty.

All of the signatories to this letter are engineering physicists who have filed affidavits regarding the various letters and articles relevant to *The Progressive* case and two of us have had access to the classified and "in camera" documents. This letter has no official connection with the Argonne National Laboratory, with the University of Chicago, nor with the UK Department of Energy.

Your faithfully,

Gᴇʀᴀʟᴅ E. Mᴀʀsʜ
Aʟᴇxᴀɴᴅᴇʀ Dᴇ Vᴏʟᴘɪ
Gᴇᴏʀɢᴇ S. Sᴛᴀɴꜰᴏʀᴅ

Argonne National Laboratory,
Argonne, Illinois

Exposing sociobiology?

Sɪʀ,—I am pleased to detect a slight shift in *Nature's* editorial predilections, from totalitarian socialism to totalitarian Mahometism. At least, as Mr Crouchback would say, they have a king and some sort of religion.

Perhaps you will now find it possible to expose the sociobiological theories as no mereappendage of Naziism, but the very emanation of Satan; whose hand can now be discerned — in the horrific decline in spirituality — to say nothing of the savage cuts in public expenditure, and insecurity of tenure in medical research jobs — that has plagued the West since the Turk was stopped at the gates of Vienna.

Your faithfully,

Cʜᴀʀʟᴇs W. Cʟᴀʀᴋ

Daresbury Laboratory, Daresbury,
Warrington, UK

A new twist for DNA?

from David R. Davies and Steven Zimmerman

IN the twenty-seven years since Watson and Crick proposed that B-DNA is a right-handed double helix, the concept of a regular DNA structure, varying only in the chemical sequence of its bases, has become one of the foundations of molecular biology. Although fibre diffraction of DNA shows the existence of at least three distinct forms, thereby demonstrating the flexible nature of the sugar phosphate backbone, the structure in solution has always been believed to be a regular B form. Occasionally, suggestions have been made that some DNAs, particularly with unusual base sequences, could adopt significantly different conformations, but the evidence has never been conclusive.

This situation has recently changed dramatically. There are now two clear examples, both deoxypolymers with simple alternating sequences, for which strong evidence exists that the conformation of the backbone in solution is also alternating. In one case, poly(dA–dT)·poly(dA–dT), the current model is still a right-handed double helix similar to B-DNA in general form. For poly (dG–dC)·poly(dG–dC), on the other hand, a remarkable left-handed structure has been proposed by Wang *et al.* (*Nature,* **282,** 680; 1979) on the basis of the elucidation of the crystal structure of the hexanucleotide $d(C-G)_3$. In this crystal two molecules are combined with Watson-Crick base pairing to form a double helical segment with remarkable internal regularity. In addition, neighbouring segments pack on top of one another to approximate an infinite helix with antiparallel strands and twelve base pairs per turn. This structure has been termed 'Z-DNA'.

In this new Z helix the conformations of the guanine and cytosine deoxynucleotides differ considerably from one another so that the repeating unit becomes a dinucleotide, unlike the B-DNA helix in which the repeating unit is a single nucleotide. The torsion angles of the bases relative to the sugars are quite different. Whereas the cytosine adopts the *anti* conformation relative to its deoxyribose as in B-DNA, the guanine adopts the *syn* conformation. The puckers of the two deoxyribose rings are also different, being C3′ endo for two internal deoxyguanosines and C2′ endo for the deoxycytidines. Finally, the conformation of the backbone about the C4′-C5′ bond is *gauche-trans* for the dG residues and *gauche-gauche* for dC. The net result of these differences between the purine and pyrimidine nucleotide conformations is to produce a staggered zig-zag course for the deoxyribose phosphate backbone, hence 'Z-DNA'.

David R. Davies and Steven Zimmerman are in the Laboratory of Molecular Biology, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland.

These single crystal results imply strongly that this hexanucleotide structure could exist in a polymer of deoxynucleotides. Indeed, Arnott *et al.* (*Nature* in the press) have now observed a form of poly(dG–dC)·poly(dG–dC) that they believe from X-ray fibre analysis to be the Z-form. The helical parameters of this fibre pattern show a remarkable correspondence to those of the crystal. The former have a helical pitch of 43.5 Å with 12 base pairs, compared with the prediction from the crystal of 44.6 Å. The fibre also provides evidence for a dinucleotide repeating unit.

Arnott *et al.* (*op. cit.*) have observed that the same fibres that initially gave B-form diffraction patterns later gave Z-form patterns. This implies that the molecules can undergo a change of direction of the helix twist within the fibre in a relatively confined space without greatly altering the orientation of the double helices and without getting significantly tangled. Wang *et al.* (*op. cit.*) point out that the transformation between Z and B will involve separation of the base pairs, a large rotation of the guanine base accompanied by a rotation of the entire deoxycytidine residue, followed by rejoining of the base pairs. They note that the junction between Z and B-DNA will have a stacking discontinuity which might result in a kink in the molecule.

There is evidence that the Z-DNA conformation is not restricted to poly (dG–dC)·poly(dG–dC). Arnott *et al.* report the observation of this form in fibres of poly(dG–dT)·poly(dA–dC). They also interpret the X-ray diffraction results of Saenger *et al.* (*Jerusalem Symposium on Quantum Chemistry and Biochemistry* **V,** 457; 1973) for the alternating copolymer of deoxyadenylate and 4-thiothymidylate in terms of a modified Z helix with 14 base pairs in a pitch of 54 Å.

The anomalous behaviour of poly (dG–dC)·poly(dG–dC) has been known for some time. Pohl and Jovin (*J. molec. Biol.* **67,** 375; 1972) were the first to observe two distinct forms of poly(dG–dC)·poly (dG–dC) in solution, with a cooperative transition occurring between them at high salt concentrations. The circular dichroism spectrum of the high salt form is nearly an inversion of that of the low salt form, which in turn resembles that of B-DNA. This suggests that the transition to the high salt form involves a rearrangement of the bases. Wang *et al.* (*op. cit.*) believe that their Z-form corresponds to the high salt form, even though the $d(C-G)_3$ was crystallised at relatively low salt concentrations. This interpretation is consistent with the results of a previous NMR investigation of $(dG-dC)_8$ by Patel *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **74,** 2508; 1979), who clearly demonstrated that the repeating unit in the high salt structure is a dinucleotide, and that marked differences between the two phosphodiester linkages (that is, dCpG and dGpC) give rise to two peaks in the high salt ^{31}P NMR spectrum. Differences in the sugar hydrogen resonances of the two forms also led to a prediction of a dramatic change in either the guanosine or cytidine glycosidic torsion angle during the transition from the low to high salt form.

How do these results relate to the 'alternating B-DNA' model recently proposed for poly(dA–dT)·poly(dA–dT), (Klug *et al. J. molec. Biol.* **131,** 669; 1979)? This model is based on the crystal structure of the tetranucleotide $d(A-T)_2$ (Viswamitra *et al. Nature* **273,** 687; 1978) which also has different nucleotide conformations, with the thymidine sugars in the C2′ endo conformation while the deoxyadenosine sugars are C3′ endo; in addition the O–P torsion angles are *gauche gauche* for the dA(3′5′)dT segments and *trans gauche* for dT(3′5′)dA. Klug *et al.* used this result as the basis for a hypothetical 'alternating B-DNA' structure for polynucleotide sequences containing alternating purines and pyrimidines. Although it has an alternating backbone, this structure is closely related to B-DNA and is quite different from Z-DNA. The NMR analysis of fragments

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of poly(dA–dT)·poly(dA–dT) 145 base pairs long supports the concept of an alternating backbone conformation with a dinucleotide repeat, the ^{31}P resonance showing a small splitting (Shindo *et al. J. biol. Chem.* **254**, 8125; 1979). Also, in a joint X-ray/NMR study on fibres of poly(dA–dT)·poly(dA–dT), (Shindo & Zimmerman, *Nature* in the press) calculated NMR resonances that are consistent with the 'alternating B-DNA' model of Klug *et al.* As the 'alternating B-DNA' structure was also anticipated to occur for poly (dG–dC)·poly(dG–dC), it is surprising that the low salt form of this polymer shows no splitting of the ^{31}P resonance.

We should point out that the various models that have been proposed for left-handed or side-by-side DNA of irregular sequence (references may be found in Crick *et al. J. molec. Biol.* **129**, 449; 1979; *News & Views* **278**, 780; 1979) are quite different from Z-DNA and from the alternating B-DNA structure, both of which seem to be a response to a regular purine–pyrimidine alternating sequence.

Is the left-handed Z-form likely to be found in DNA *in vivo*? Where there are stretches of alternating GC sequence (or possibly even some other kinds of alternating purine–pyrimidine sequences), the potential will exist for forming this structure. However, the transition from B to Z occurs at the rather unphysiological salt concentrations of 2.5 M NaCl or 0.7 M MgCl$_2$ (Pohl & Jovin *op. cit.*) which might seem to make the Z-form unlikely except in halophiles; nevertheless, complex ions such as polyamines or specific binding proteins might favour the transition. It is interesting that the association of complementary closed circular single-stranded DNA molecules can occur over as much as 70% of the length of each DNA strand (Stettler *et al. J. molec. Biol.* **131**, 21; 1979). Topological considerations and the observed circular dichroism changes suggest that some segments of this DNA are left-handed but the extent, regularity and conformation of these segments are not known. If left-handed helical regions do occur in DNA *in vivo*, they might be associated with significant biological effects through their interactions with DNA-specific enzymes and binding proteins. Also, the presence of the Z-conformation in supercoiled DNA would influence the interpretations of supercoiling. It has been suggested by Gellert and Mizuuchi that the energy stored in supercoiled DNA might cause appropriate alternating sequences to snap over into the Z form even at physiological salt concentrations. On the basis of the detailed structural information now becoming available, experiments can be designed to test these possibilities. ☐

Quick-freezing — the new frontier in freeze-fracture

from David Shotton

A TECHNIQUE for the ultrastructural preservation (fixation) of biological specimens by extremely rapid freezing in the absence of cryoprotectants has been developed for freeze-fracture and thin-section electron microscopy by John Heuser (of the Department of Physiology at the University of California Medical School in San Francisco) and Thomas Reese (of the National Institute of Neurological and Communicative Disorders and Stroke, at the National Institutes of Health in Bethesda). This promises to open a new chapter in biological ultrastructure research, because it now makes possible electron microscopic studies of biological events which occur within the millisecond time range, permits the observation after freeze-etching of surface features previously obscured by non-volatile chemical cryoprotectants, and allows a critical evaluation to be made of the artefacts produced in biological specimens by the chemical fixation and cryoprotection techniques routinely used by electron microscopists.

Results which Heuser, Reese and their colleagues have obtained by using the quick-freezing technique on three distinct biological systems have recently been published in three beautifully-illustrated papers contained in the latest volumes of the *Journal of Cell Biology* (Heuser *et al. J. Cell Biol.* **81**, 275; 1979; Heuser & Salpeter *J. Cell Biol.* **82**, 150; 1979; Chandler & Heuser *J. Cell Biol.* **83**, 91; 1979), giving us a chance to assess the significance of this development.

In essence the technique is very simple, and involves bringing a biological specimen, fixed beneath the lower end of a vertical falling rod, rapidly into firm contact with the upper polished surface of a pure copper block cooled from below to approximately 4 K (–269°C) with liquid helium. Their first quick-freezing machine, described by Heuser, Reese and Landis in 1976 (*Cold Spring Harbor Symp. quant. Biol.* **40**, 17), was developed from the design originally put forward in 1964 by Van Harreveld and Crowell (*Anat. Rec.* **149**, 381; Van Harreveld *et al. J. Cell Biol.* **25**, 117; 1965). It resembled in principle the simple liquid nitrogen-cooled copper block freezing method of Dempsey and Bullivant (*J. Microscopy* **106**, 251; 1976), although it had the important advantages of automation, a colder initial block temperature and a sophisticated specimen mounting system which minimised contact damage.

An improved version of their apparatus is now described by Heuser *et al.* (*op. cit.* 1979). With it they could freeze the surface 10 μm layer of living specimens within 2 ms, with excellent ultrastructural preservation and minimal ice crystal damage. Further from the surface, the inherently low thermal conductivity of biological tissue limited the rate of heat loss and unavoidably led to the growth of large ice crystals and consequent tissue damage, similar to that observed whenever tissue is frozen without previous chemical cryoprotection by conventional techniques of immersion into freezing Freon-22 or nitrogen slush.

Once frozen by the quick-freeze method, specimens can either be freeze-fractured and replicated in the normal way, care being taken to ensure that the fracture plane passes through the surface 10 μm layer, or alternatively can be freeze-substituted and chemically fixed at –95 °C in a thawing acetone-osmium tetroxide mixture before embedding for thin-section electron microscopy. One particular advantage of this copper block quick-freezing method over liquid propane jet-freezing methods, which also give improved rates of cooling, is that the well-frozen layer of the specimen is physically flat and parallel to the specimen support, enabling extensive freeze-fracture replicas from this region to be obtained using a Balzers microtome system.

The most important and exciting advantage of this quick-freezing method, in comparison with conventional ultrastructural approaches, is that it enables living specimens to be instantaneously frozen with good ultrastructural preservation at known intervals after a biological stimulus, and so makes it possible to use the electron microscope for kinetic studies of transient biological events which are completed within a few seconds or even, with certain favourable specimens, within a few milliseconds. It was for this purpose — to visualise the train of events which occurs at the presynaptic membrane of the frog neuromuscular junction during the first few milliseconds after the arrival of a nerve impulse — that Heuser and Reese originally developed their apparatus. The results they obtained are presented in the papers by Heuser *et al.* (1976 and 1979, *op. cit.*) and Heuser *Soc. Neurosci. Symp.* **2**, 215; 1977), and a further paper now in press.

Living cutaneous pectoris muscles were dissected intact from frogs, with their motor nerve attached. Each thin sheet-like muscle was individually mounted onto the specimen support of the quick-freezing machine, and was caused to contract by

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David Shotton is a Lecturer in the Department of Biochemistry, Imperial College, London.

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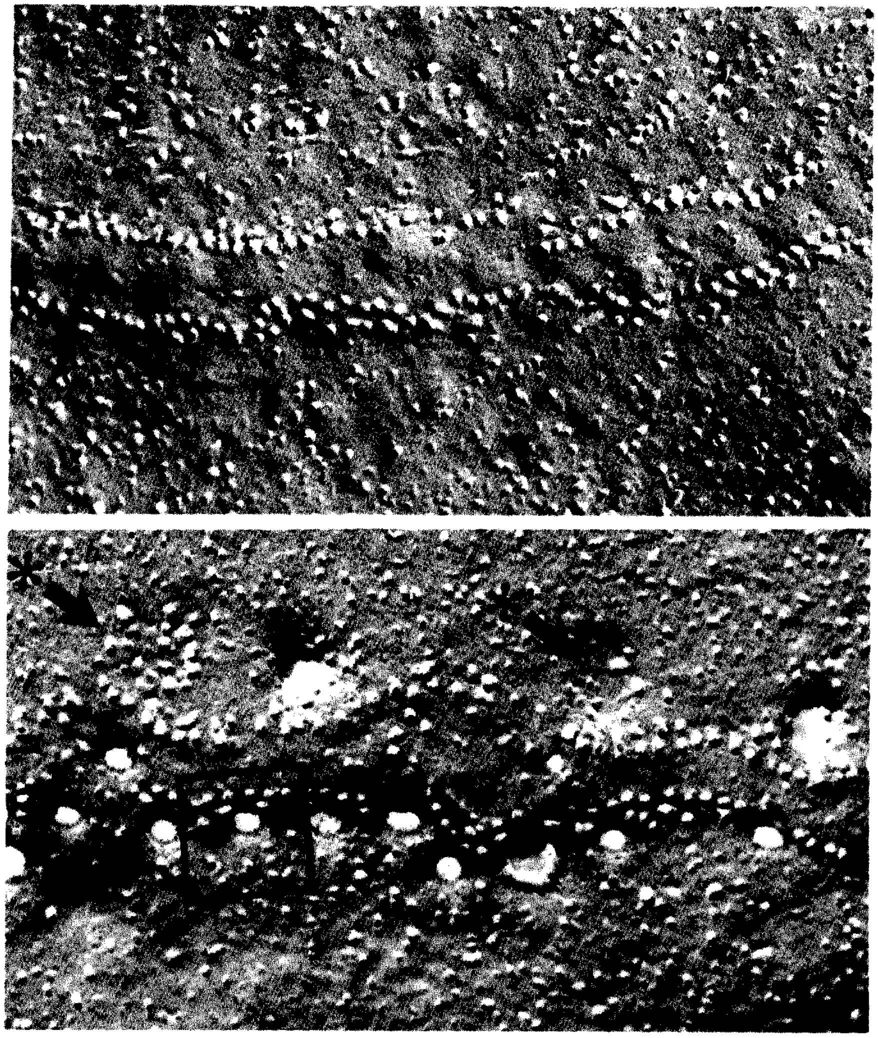

Fig. 1 *a*, High magnification view of the 'P face' of one active zone from a frog motor nerve terminal soaked in 1 mM 4-AP and 10 mM Ca^{2+} for 30 min and given one stimulus 3 ms before freezing. By 3 ms, the action potential presumably arrived in the terminal, but it did not alter the resting appearance of the active zone, which is recognised as a slight ridge bordered by parallel rows of large intramembrane particles. × 143,000. *b*, P-face view of an active zone from a nerve terminal treated like the one in 1*a*, but given one nerve stimulus 5 ms before freezing. In the interval between 3 and 5 ms, just at the time when the nerve begins to discharge large numbers of acetylcholine quanta, many membrane perturbations appear along the edges of the active zone. These are believed to be exocytotic stomata, or openings into the underlying synaptic vesicles. The two areas marked by asterisks are thought to be vesicles that have collapsed flat after opening; they display a telltale cluster of two or more large intramembrane particles like those found in intact synaptic vesicle membranes. The square encloses an early stage in one characteristic exocytosis event.

electrical stimulation of the nerve as it fell towards the cold copper block. The interval between the arrival of the nerve impulse at the neuromuscular junction and the moment at which the specimen froze solid was recorded on an oscilloscope, and was later related to the appearance of the presynaptic membrane after that specimen had been freeze-fractured, enabling the investigators to determine the time course of the exocytotic events caused by the fusion of the synaptic vesicles with the presynaptic membrane. 4-aminopyridine (4-AP) was used to increase the number of acetylcholine neurotransmiter quanta discharged with the arrival of each nerve impulse from the normal level of less than one quantum per presynaptic active zone of transmitter release to a level at which they could more easily be studied. No structural changes were observed during the first 3 ms after stimulation (Fig. 1*a*), but by 5 ms narrow exocytotic openings were visible at fusion sites all along the active zones (Fig. 1*b*). During the subsequent 20 ms, these fused vesicle membranes collapsed and flattened out, leaving only faint depressions in the postsynaptic membrane, each containing two or more particularly large intramembrane particles not previously present on the presynaptic membrane. The subsequent fate of the vesicle membrane components thus inserted into the presynaptic membrane was followed by quantifying the movement of these large intramembrane particles over the next 400 ms, during which period they moved away from the active zones towards sites of coated vesicle endocytosis, in the region between adjacent active zones.

The ability to undertake such direct kinetic studies represents a significant breakthrough in cell biology, since in the past kinetic sequences of rapid events at the ultrastructural level could only be deduced from static electron micrographs of chemically-fixed specimens, arranged into postulated temporal sequences on the basis of the investigator's preconceptions of what might reasonably be expected to occur. For neurobiology in particular, these studies provide further strong direct support for the synaptic vesicle fusion and recycling scheme first proposed by Heuser and Reese in 1973 (*J. Cell Biol.* **57**, 315).

The second advantage of quick-freezing is that, by being able to freeze delicate biological specimens from dilute aqueous solutions in the absence of chemical cryoprotectants without ice crystal damage, one can then expose the outer surfaces of membrane, extracellular structures and intracellular cytoskeletal elements by freeze-etching, a technique in which water molecules are allowed to sublime from the frozen surface of the fractured specimen before replication. In the past this technique could only be applied with any degree of success to rather sturdy small cells in suspension, such as erythrocytes, yeast and bacteria, which could withstand conventional freezing procedures without cryoprotection.

Heuser and Salpeter (1979 *op. cit.*) have studied unprotected quick-frozen postsynaptic membranes from the electric organs of the electric rays *Torpedo californica* and *Narcine brogiliensis* in this way. Besides giving us fascinating views of the structure of the basal lamina within the synaptic cleft, of intracellular cytoskeletal filaments and of synaptic vesicles, they have revealed that the acetylcholine receptor molecules form striking extensive ordered arrays, which can be seen not only as protrusions on the etched external surface of the postsynaptic membrane (Fig. 2*a*), resembling those previously reported on etched *Torpedo* membrane vesicles by Cartaud and his colleagues (*FEBS Lett.* **33**, 109; 1973; *Proc. 6th European Congress on Electron Microscopy, Jerusalem*, 336; 1976; *J. Cell Sci.* **29**, 313; 1978) but also as low-profile particles on the E fracture face of these unfixed quick-frozen membranes (Fig. 2*b*). Orci, Perelet and Dunant (*Proc. natn. Acad. Sci. U.S.A.* **71**, 307; 1974) had previously obtained limited views of such E-face particle arrays, but in others' hands they have usually been rendered invisible by artefactual changes resulting from glutaraldehye fixation and glycerination. They occur at a density (>10,000 μm^{-2}) which correlates well with previous physiological and autoradiographic estimates of the receptor packing density. This density is much higher than that of the larger intramembrane particles seen on the fracture faces of both fixed and unfixed membranes, which had often previously been assumed to represent the receptor molecules.

The third advantage of the quick-freezing method is that it enables one to evaluate the artefacts produced in biological specimens by the alternative chemical fixation and cryoprotection techniques normally used by electron microscopists. One example of this, from the work of Heuser and Salpeter, is the striking differences in appearance and aggregation state of the intramembrane particles on the P and E fracture faces of *Torpedo* postsynaptic membranes when freeze-fractured after quick-freezing and after glutaraldehyde fixation, glycerination and Freon freezing. Other examples of such artefacts, which are important in assessing current theories of membrane fusion, come from the studies which Chandler and Heuser (*op. cit.* 1979) have made of cortical granule exocytosis in fertilised sea urchin eggs. In this work, the authors used the quick-freeze technique to capture stages in the 'cortical reaction' which originates from the point of entry of the sperm and which within 30 s sweeps across the entire surface of the egg. This process starts with the explosive exocytosis of the cortical granules, causing the characteristic elevation of the vitelline layer, and is followed by an elaboration of microvilli and subsequent membrane recovery by coated vesicle endocytosis. Chandler and Heuser found that freeze-fracture replicas of eggs fixed with glutaraldehyde and cryoprotected with glycerol before freezing displayed forms of interaction between the granule membrane and the plasma membrane, including particle-free single-bilayer membrane diaphragms formed from apposed fusing membranes, which looked identical to those described by other investigators and proposed as essential intermediates in the process of membrane fusion (see *News and Views* **272,** 16; 1978). However such membrane interactions were never found in eggs which had been quick-frozen without glycerination, even after glutaraldehyde fixation. The authors review other recent literature reporting lability of aldehyde-fixed membranes and glycerol-induced membrane changes, and conclude that many of the postulated 'intermediate stages' in fusion are in fact artefacts resulting from the lack of complete stabilisation of lipid bilayers by aldehyde fixation, exacerbated by the dehydrating effects of glycerol. This is further discussed by Chandler in a forthcoming book entitled *Freeze-fracture: Methods, Artifacts and Interpretations* (eds Rash & Hudson, Raven Press, New York). Since glutaraldehyde does not affect most membrane lipids, such lability is not unreasonable, but the question remains as to what chemical changes have already occurred in those membranes to permit such artefacts to develop only in situations where membrane fusion is imminently likely to occur.

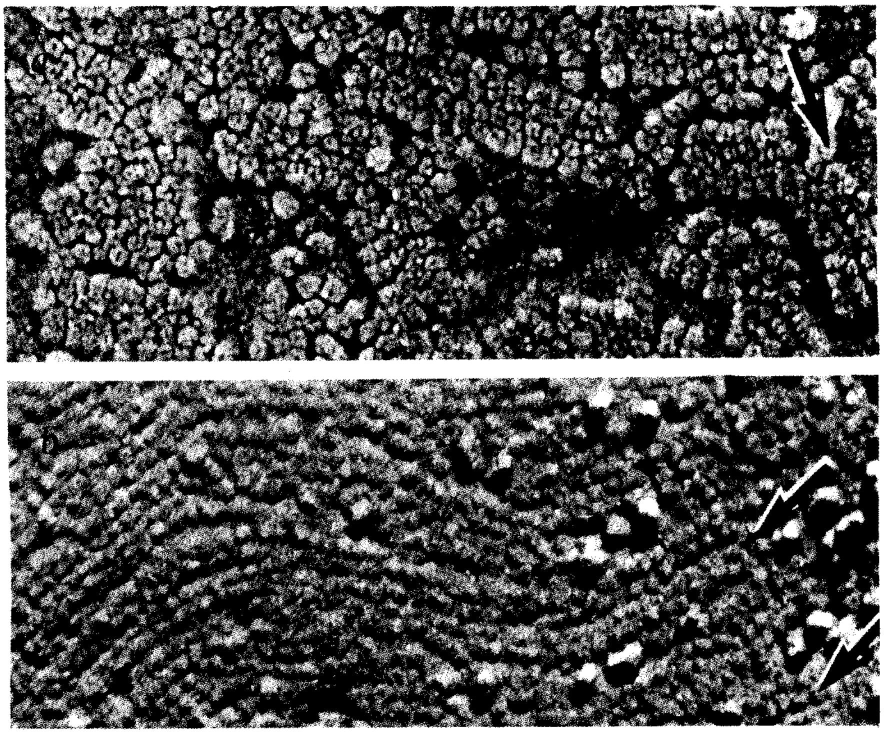

Fig. 2 *a,* Characteristic view of the pattern of grouping of surface projections seen on deep-etched postsynaptic membrane fragments. The most obvious pattern is rows four abreast, with many breaks and curves (arrow). × 270,000; so 3 mm ≃ 10 nm. *b,* Unidirectional shadow-casting of an unetched E face of the postsynaptic membrane, exposed by fracturing intact, quick-frozen tissue, which illustrates that the shallow bumps seen on this surface also group into curvilinear rows, most often two or four abreast (arrows), like the surface projections in Fig. 2a × 270,000; so 3 mm ≃ 10 nm.

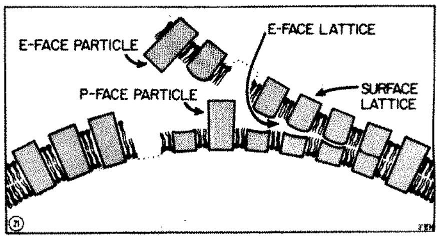

Fig. 3 Diagram illustrating, first, how cleavage of intramembrane receptor molecules could generate the match between the E face lattice and the surface lattice of projections that is observed in etched *Torpedo* post-synaptic membrane; and second, how extraction of whole receptors from one leaflet or the other could generate the large intramembrane particles which also characterise this membrane.

The quick-freezing method instantaneously samples one particular moment in a biological process, and avoids the accumulation of intermediate stages which sometimes happens during slow death aldehyde fixation. As Chandler and Heuser were not able to detect preliminary fusion stages in their quick-frozen fertilised eggs, before seeing fused granules open to the extracellular space, they concluded that such stages could last no longer than 5 ms. An understanding of the molecular events which initiate membrane fusion thus seems as elusive as ever! ☐

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100 years ago

The *Hannoversche Courier* announces that Leibnitz's long-lost calculating machine has been recovered. Leibnitz invented and constructed this machine in 1672, during his stay in Paris. It can add, subtract, divide and multiply and was the wonder of the time. This machine became the property of the Hanover public library, but long ago disappeared from among its treasures. All that was known about its disappearance was that it had once been sent to an instrument maker at Göttingen to be repaired. It has now turned up again in the Göttingen library.

It is only about a year since we gave some account (Nature, vol. xviii. p. 361) of the railway bridge which spans the Firth of Tay at Dundee, and on Sunday it was the scene of one of the most terrible railway accidents on record. With the details of this sad occurrence our readers are no doubt familiar; for accurate information as to the prime cause we must await the searching inquiry which will no doubt be instituted. The structure appears to have been subjected to the most rigid tests before being opened to traffic, but we fear there must have been more than one screw loose somewhere. Upwards of 3,000 feet of the high girders are reported to have been swept away. One conjecture is that the train had got well upon the girders when a gust of greater strength had caught the structure. There would thus be, in addition to the ordinary vibration of the train, an enormous lateral pressure from the wind.

From *Nature* **21,** Jan. 1, 214; 1880.

The great mouse festival

from R.J. Berry

To most people the house mouse is a commensal nuisance; to many biologists it is a necessary research tool, bought off the shelf (as it were) in the same way as a chemical. Yet the mouse is a real animal, approved because "its fertility, prolificity, convenient size, short gestation period, its manifold variations, inexpensive maintenance, resistance to open infections, susceptibility to certain diseases, and ease of production conspire to make it the laboratory animal par excellence". So wrote Clyde Keeler in 1931. Ten years later, L.C. Strong was advocating that "... within the near future all research on mice should be carried out on inbred animals or on hybrid mice of known (genetically controlled) origin, where the degree of biological variability has been carefully controlled". As immunological studies developed, this became essential; but as a consequence laboratory mice became more and more removed from their origins.

There are now about 200 highly inbred strains of mice. However the great bulk of these arose from a very narrow base — notably the mouse colony established in Granby, Massachusetts during 1903-15 by Miss Abbey Lathrop, who supplied, among others, W.E. Castle and C.C. Little. The origins of her mice are not known in detail but they included 'fancy' mice imported from various European countries and wild animals from Vermont and Michigan, which were crossed to them. Such widely used strains as the C57 group are descended from the Lathrop colony.

The genetical poverty of established laboratory strains as a result of their small number of ancestors has led in recent years to an interest in wild mice as a source of new inherited variants: the first mouse inborn error of metabolism analogous to a human anomaly (histidinaemia) was discovered in a mouse from Peru; several mice caught five years ago in Taunton were found to have a similar defect in pyruvate kinase to one which produces a clinical anaemia in man. More recently mice from south east Asia (notably *M.m. castaneus* and *M.m. molossinus*) have proved particularly rich in previously unknown variants.

An older line of research in wild mice stems from the widespread occurrence of *t*-alleles in natural populations. Work on the *t*-complex has been stimulated in the past few years because of its linkage to the major histocompatibility complex (*H-2*), with suppression of crossing-over between the two chromosomal segments if a *t*-allele is present. It has been suggested that the *t* and *H-2* segments may represent a duplication, *t* acting as a tissue recogniser before birth and *H-2* taking over post-natally.

Finally, mice have been important to evolutionists because of their apparently very tight social structure, from which it has been argued that the 'effective population size' of mouse populations is less than four individuals, thus permitting considerable genetic drift between generations and providing a paradigm for testing ideas of neutrality and selection acting on particular inherited variants.

All these interests found a focus in a symposium on the house mouse held in London recently*. It reviewed developments over a wide range of fields from archaeology and taxonomy to gerontology and zoonoses. As such it established hybrid vigour between different disciplines rather than transitory euphoria from exciting experiments. The recurring theme was the problem of identifying the stresses and stratagems of animals exposed in different parts of their range to widely differing environmental conditions. R.J. Berry (Royal Free Hospital Medical School, London) described gene frequency changes of over 15% between cohorts of mice in the same populations living on sub-Antarctic islands, with the two sexes showing changes in opposite directions on South Georgia. No changes occurred in populations living on tropical Pacific islands. But what do these changes mean? J. Klein (University of Tübingen) suggested that the levels of heterozygosity (over 95%) found at the major histocompatibility locus *H-2* (Duncan *et al. Nature* **281,** 603; 1979) are the result of selection by pathogens acting on varying gene pools established by small numbers of founders. This interpretation depends to some extent on a deme structure of mouse populations allowing local differentiation, and the hitherto generally accepted tightness of this structure was challenged from the point of view of ethological and chemical interactions between individuals by J.H. Mackintosh (Birmingham) and Jane Smith (Pest Infestation Control Laboratory, MAFF).

Implicit in all these approaches was the recognition that both an individual mouse and a mouse population changes in time. This was spelt out by D. Bellamy (Cardiff) who emphasised that many influences are involved in ageing, and it is dangerous to assume that a wild mouse which reaches its physical peak at around 100 days and dies at 400-500 days (even in the laboratory) is responding to the same processes as an inbred animal which may live up to 800 days. A similar point was made by M.E. Jakobsen (North East London Polytechnic) in criticising the naivety inherent in many laboratories studies of physiological responses (particularly brown fat amount and turnover) when compared with the complex and variable interactions of behaviour and time in mice living in nature.

Notwithstanding, one of the values of the house mouse is the possibility of feedback between knowledge gained of animals living under wild conditions and those kept under controlled conditions in the laboratory. Mary Lyon (MRC Radiobiology Unit, Harwell) reviewed the complexity of gene action in the supergenes on chromosome 17, where embryological analysis of the effects of different loci is a necessary complement to Klein's immunological work. The potential of laboratory animals for interpreting the wild situation (and *vice versa*) was also apparent in contributions from R.C. Roberts (ARC Unit of Animal Genetics, Edinburgh) on the determinants of growth (strong selection for small size in laboratory mice failed to reduce them to the mean size — around 9 gram — of animals from the tropical Pacific) and Jo Peters (MRC Harwell) on protein variation, while J.G.M. Shire (Glasgow) showed how laboratory studies on wild caught mice had influenced — and could enrich — our understanding of endocrine action by providing new structural variants of hormones and by affecting target organ sensitivity.

Surprisingly, the most radical papers came from two taxonomists, J. Marshall (US Natural History Museum) and L. Thaler (Montpellier) who broke the news that the unitary widespread Linnaean species *Mus musculus* with four recognised and 164 invalid subspecies is no more. Thaler spoke merely of species nos 1, 2, 3 and 4 in Europe, but Marshall went the whole taxonomic hog and gave us *Mus musculus, domesticus, spretus* and *abbotti* (=*spicilegus*) in western Europe, *hortulanus* in eastern Europe, *molossinus* in Japan, and *castaneus* as an Asian commensal.

But house mice are even more complicated: traditionally they have had 20 pairs of very uniform acrocentric chromosomes, but local races with 11, 13 and 14 pairs of chromosomes due to centric fusion are now known, and these naturally occurring chromosomal anomalies can be used to produce aneuploid mice, and hence study the pathological effects of aneuploidy (A. Gropp, Lubeck). A new Robertsonian fusion (with 38 chromosomes) from an Orkney island was reported by Klein. No-one knows why these chromosomal races have arisen, and why they persist.

The implications of our knowledge of house mice for ecologists are great: we know more about the behaviour and

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R.J. Berry is Professor of Genetics at the Royal Free Hospital Medical School, London.

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*A symposium on the 'Biology of the House Mouse' was held at the London Zoo on November 22-23, 1979.

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genetics of this species(s) than any other. But the real significance of house mouse biology is for biomedical workers: their inbred strains ought no longer to be regarded merely as biochemical artefacts, but as interacting feedback systems dependent on an evolutionary history of adaptation and opportunism. It is pertinent that 13 of the 22 speakers at the Zoo Symposium were employed in Medical Schools or by the MRC.

As medical resources become increasingly stretched, they will have to be applied more consciously at points of need rather than in blunderbuss endeavours to improve the 'environment' (such as water quality, toxicological and dietary hazards, or prenatal care). Wild house mice provide a logical step in interpreting results obtained in the artificiality of the laboratory environment to the confusing heterogeneity of human life. It is to be hoped that the symposium will stimulate both research and funding for work on naturally-living mice. ☐

Why ppGpp?

from Andrew Travers

THE ubiquity of purine nucleotides as regulators of macromolecular synthesis was highlighted last summer at a workshop most appropriately dedicated to Fritz Lipmann*. Historically the identification of regulators such as cyclic AMP, ppGpp, pppAppp, A$^{5'}$ pppp$^{5'}$ A and 2′5′ oligo-A has depended on their unusual and often bizarre structutes. Yet in evolution such control systems are unlikely to have arisen *de novo*. Rather it seems more probable that they initially evolved as more effective analogues of pre-existing systems. Such analogues should necessarily be chemically similar to the primordial regulators yet should possess some distinguishing structural features which would allow greater precision in the control of their intracellular concentration.

ppGpp may be considered as a paradigm of such an effector. Its accumulation in bacteria is elicited both by energy starvation and amino acid starvation and, in general, is accompanied by the reduction of protein and RNA synthesis to basal levels. Both genetic (A. Atherley, Iowa State University, Ames; M. Cashel, National Institutes of Health, Bethesda) and biochemical analysis (D. Richter, Universität Hamburg; J. Sy, Rockefeller University) indicate that there are two pathways for its synthesis, one dependent on ribosomes and activated by uncharged tRNA and a second whose activity is apparently independent of protein synthesis. Similarly the degradation of ppGpp to either ppGp or ppG may be equally complex (Richter).

What is the role of ppGpp? The isolation of a viable mutant which lacks detectable ppGpp clearly demonstrates that the nucleotide is dispensable during normal growth (Cashel). However the ability of the mutant to undergo growth transitions is somewhat impaired. One correlation with the accumulation of ppGpp consequent on environmental stress is a change in transcriptional selectivity manifested as a preferential shut-off of stable RNA production. *In vitro* physiological concentrations of the nucleotide inhibit the initiation of rRNA synthesis with either promoter bound or free RNA polymerase as its target (M. Gruber, Rijiksuniversiteit, Groningen; A. Travers, MRC Laboratory of Molecular Biology Cambridge). Yet the extent of inhibition of rRNA synthesis *in vitro* is insufficient to account for the *in vivo* effect. Either the *in vitro* system lacks an activator normally present *in vivo* or ppGpp is not the immediate effector of transcriptional changes following amino acid starvation. Indeed a minor guanine nucleotide, ppGp, was proposed as a candidate true regulator (J. Gallant, University of Washington, Seattle).

Although most evidence favours the hypothesis that ppGpp is a major regulator of macromolecular synthesis during nitrogen starvation analogous changes in gene expression can occur without the concomitant accumulation of this nucleotide. This phenomenon is seen in strains containing mutations in the gene encoding fructose 1:6 diphosphate aldolase (Atherley; Bock & Neidhardt *J. Bact.*, **92**, 470; 1966). This observation again points to the possibility that the response of transcription to environmental stress depends on the balance between the levels of a number of effectors. Such a mechanism would allow diverse means of eliciting the same response. One example of this phenomenon is the process of sporulation in *Bacillus subtilis*. This can be induced by either nitrogen or energy starvation. The latter results in a substantial increase in the intracellular concentration of an adenine nucleotide tentatively identified as pppAppp (H. Rhaese, Universität Frankfurt). By contrast, on nitrogen starvation the GTP pool falls without a concomitant increase in pppAppp levels (E. Freese, National Institutes of Health, Bethesda). In both cases the correlation between the observed phenomena and the physiological response is strong and suggests a causal effect. To reconcile such apparently disparate observations it has to be argued that a fall in the GTP pool is equivalent to a rise in the pppAppp pool. This would be compatible with the opposing functional effects of adenine and guanine nucleotides on RNA polymerase.

Although the appearance and immediate effects of regulatory nucleotides such as ppGpp and pppAppp may be transient there is much evidence to suggest that their long term effects may also be important. The accumulation of ppGpp is accompanied by the preferential synthesis of two proteins, B56.5 and stringent starvation protein (Reeh *et al. Molec. gen. Genet.* **149**, 279; 1976). At least the latter binds to RNA polymerase and seems to alter its properties in a similar manner to ppGpp itself (Ishihama & Saitoh *J. molec. Biol.* **129**, 517; 1979). Thus ppGpp may induce the synthesis of proteins which act to stabilise the effect which the nucleotide itself elicits. Such a process would constitute a mechanism for changing the state of the cell. In a like manner the 37,000 molecular weight polymerase binding protein which appears in the initial stage of sporulation in *B. subtilis* (Haldewang & Losick *Nature*, **282**, 256; 1979) could stabilise the effects of the transient perturbation of the adenine and guanine nucleotide pools.

In eukaryotes, with the exception of cyclic AMP, there is little evidence for selective regulators of gene expression analogous to ppGpp. In yeast changes in nucleotide pools similar to those observed in *B. subtilis* are correlated with sporulation (Freese; Rhaese). Yet in higher eukaryotes ppGpp and similar compounds are apparently absent (R. Silverman, Iowa State University). Nevertheless highly phosphorylated compounds were reported to accumulate after heat shock in *Drosophila* (Travers) and immediately before sporulation in the water mould *Achyla* (H. Lejohn, University of Manitoba, Winnipeg). Although the appearance of these compounds correlates with changes in macromolecular synthesis there is as yet no conclusive evidence as to whether they have a regulatory role. However adenine nucleotides have been implicated as signals for the initiation of DNA replication. One proposed universal regulator is A$^{5'}$ pppp$^{5'}$ (P. Plesner, Odense University), which binds tightly to a single subunit of DNA polymerase α (F. Grummt, Max-Planck Institut für Biochemie, München). Alternatively, the effective signal could be the balance between ATP and ADP in the cell nucleus (E. Rapaport, Harvard University). Such a mechanism would utilise purine nucleotides required for essential intracellular processes, a characteristic which might also be expected to typify early stages in the evolution of regulatory processes.

If adenine nucleotides act as indicators of the energy balance within the cell what is the evolutionary origin of the guanine nucleotide regulators? In bacteria the major physiological role of guanine nucleotides is their involvement in protein synthesis which generates GDP from GTP. ppGpp and related nucleotides could thus be derived from a primitive regulatory system utilising the simple 5′ di and triphosphates for coupling transcription and translation. ☐

Andrew Travers is in the MRC Laboratory of Molecular Biology, Cambridge.

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*Workshop on Low Molecular Weight Mediators of Macromolecular Metabolism was held on 24-27 July, 1979 in Hamburg and organised by G. Koch and D. Richter.

ARTICLES

Scenario for a warm, high-CO_2 world

T. M. L. Wigley, P. D. Jones & P. M. Kelly

Climatic Research Unit, University of East Anglia, Norwich, UK

Plausible patterns for temperature and precipitation changes accompanying a general global warming, such as might occur due to a large increase in atmospheric carbon dioxide levels, are presented. The patterns are determined by comparing the five warmest years in the period 1925–74 with the five coldest in this period. Temperature increases are indicated for most regions, with maximum warming over northern Asia. A few isolated regions show cooling. Precipitation changes are fairly evenly distributed between increases and decreases; the most important features being an increase over India, and decreases in central and south-central USA and over much of Europe and Russia. The latter decreases, should they occur, could have considerable agricultural impact.

MAN has upset the global carbon cycle by burning fossil fuels and, probably, by deforestation and changing land use. The net result of these activities is to increase the CO_2 content of both the atmosphere and the oceans. Although some details of the carbon cycle remain to be determined[1], it is generally believed that if fossil fuel use continues to increase at about the rate of increase experienced over the past century then atmospheric CO_2 levels will reach roughly twice today's level by the middle of the next century[2,3]. Furthermore, the enhanced greenhouse effect, could well cause a significant increase in global mean annual surface temperature. Numerical modelling experiments suggest that this increase would be 2° to 3 °C (refs 4–6), a change as great as or greater than any which has occurred in the past 10,000 years. The magnitude of the increase is, however, uncertain and a CO_2-induced warming could possibly be offset by a natural cooling trend and/or by the effects of various feedback mechanisms (such as that due to possible changes in cloud cover).

Although there is some reason to doubt that a significant warming will occur, its consequences are so great that the possibility must be considered seriously. We will, for the purposes of this article, assume that a global warming will occur. If it does then relatively large shifts in the main atmospheric circulation systems would accompany such a change. These circulation changes will determine the geographical patterns of the changes in temperature, precipitation and winds. Although most regions are expected to warm, the amount of warming must vary considerably from place to place[7], and it is possible that some regions will become cooler; and although global average precipitation is expected to increase[8–10] some parts of the world will most probably become drier[8,9]. To assess the impact of these changes on society and decide on possible ameliorating courses of action, one must evaluate impacts on a regional scale. It is therefore essential to have some idea of the spatial distribution of future changes in climate. We present here a meteorologically and climatologically self-consistent (that is 'realistic') scenario for the pattern of changes based on recent instrumental records of the natural year-to-year fluctuations in climate.

Insights into a warm world

Two approaches may be used to derive a scenario for the pattern of climatic changes which might result from a large increase in atmospheric CO_2. These are: numerical modelling using general circulation models (GCMs)[10–12]; and the use of past warm periods as analogues of the future[8,9]. The latter includes the possibility of using recent instrumental data to determine the characteristics of individual warm years which may then be used as analogues of the future.

Both methods have their limitations. GCMs are restricted by their present state of development; current computer power dictates that these models simulate in detail only one part of the atmosphere–hydrosphere–cryosphere system. Most models only consider the atmosphere and use the hydrosphere and cryosphere as externally specified boundary conditions[11]. GCMs do, however, simulate present-day climate reasonably well; and, provided sea-surface temperatures and ice margins are prescribed, they also appear to simulate ice-age climate in a realistic way[11,13]. For a high-CO_2 world we cannot accurately prescribe sea-surface temperatures and should ideally use a coupled ocean-atmosphere model. Although such models do exist, their simulation of the thermodynamics and dynamics of the oceans is still at a rudimentary level.

Although there is agreement on many of the general results for the high-CO_2 world which have been estimated using GCMs (and other, simpler models), the only spatial detail which has been estimated is the latitudinal variation of changes in temperature[4,6,10]. These results indicate that the greatest temperature changes will occur at high northern latitudes (particularly above 60°N) and in winter. In these respects, expected CO_2-induced temperature changes show a similar dependence on latitude and season as do the 'natural' fluctuations which have occurred this century: maximum natural variability occurs in winter and in the latitude band 50 to 70°N[14,15].

The second method is to use the past as an analogue for the future. Four possible candidates have been described by Flohn[16–18]: the mediaeval warm period (about AD 800–1200), the Holocene warm period known as the Hypsithermal or Altithermal (around 4,000 to 8,000 yr BP), the last (Eemian) interglacial (isotope stage 5e, about 120,000 yr BP), and the last period when the Arctic Ocean was believed to be ice free (before 2.5×10^6 yr BP). These times are possible analogues for progressively warmer future climates. As Flohn points out, the extent to which these times may be useful as analogues depends in part on how well the ocean and cryosphere boundary conditions which occurred in the past compare to those which might occur in the future. In all four cases, boundary conditions were increasingly different from today. A further difficulty in using any of these periods as analogues is that the available data are quite sparse and are generally indirect and qualitative.

The most detailed analysis of a past epoch as an analogue for a future warmer world is Kellogg's[8,9] analysis of the Hypsithermal. (Sarnthein[19] has also made a detailed study of this period, although not as a warm world analogue.) Kellogg has used published interpretations of pollen data to compile a picture of summer precipitation patterns relative to the present.

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Unfortunately, these data are often poorly dated, and what appears to be a synchronous spatial pattern may be the superposition of a number of episodes which all occurred at slightly different times. Nevertheless, Kellogg's picture of global precipitation during the Hypsithermal is widely quoted as an analogue for a high-CO_2 world.

The recent past, for which instrumental records are available, may also be used as a guide to establishing possible future patterns of climatic change. The mean annual surface temperature of the Northern Hemisphere (and quite probably of the whole globe) varies considerably from year to year. Because of this variability one can select individual warm years as analogues for a future warmer world (resulting from increased CO_2, or other causes); or, alternatively, use a composite of a number of warm years as a warm world analogue. We have used this method in the present study. A similar approach has been used by Williams[20], Namias (unpublished) and Drozdov[21] (although this latter work was not directed towards the CO_2 issue.)

The use of any past records as analogues for a future high-CO_2 world can be criticised on the grounds that past climatic fluctuations were not caused by changes in atmospheric CO_2 content. Although the causes of year-to-year fluctuations in this century are not clearly understood, CO_2 is not a possibility. In defence against this criticism we note that numerical model results indicate that, whatever the cause of the warming, there are broad similarities in the patterns of climatic change. The computed effects of, for example, an increase in the solar constant[12] are similar to those due to increased atmospheric CO_2[4]. Furthermore, as noted above, what little is known about the latitudinal and seasonal variations in temperature changes which might result from increased CO_2 indicates that these variations are similar to those for natural year-to-year temperature changes. Essentially, in making use of natural fluctuations we are assuming that, given similar boundary conditions, there are broad similarities in the way the atmosphere responds to different types of forcing.

Scenario for a high-CO_2 world

Instead of comparing a group of recent warm years with the long-term mean in order to derive a warm-world scenario, we have used the variations in mean annual surface temperature to maximum advantage by comparing a composite of the five warmest years in the period 1925–74 with a composite of the five coldest years in this period. The period 1925–74 has been chosen because, although temperature data exist before 1925, there are substantial gaps in the coverage and problems of comparability of early and more recent records which make it difficult to rely on the values of, and changes in, spatial means and patterns.

We have chosen to define 'warmest' and 'coldest' in terms of the latitudinal zone 65° N to 80° N. This choice is based partly on data availability, but also on the generally accepted belief that CO_2-induced changes will be greatest in high northern latitudes. The use of high-latitude data eliminates many of the large data-poor ocean areas. To facilitate averaging over arbitrary regions of the globe, and to produce a homogeneous data series over the whole period, we calculated mean temperatures by first calculating grid point temperatures from station data. Our methods differ slightly from other authors, but our results correlate highly with those of Budyko[22], Reitan[23], Angell and Korshover[24] and Borzenkova *et al.*[25].

For the 65° N to 80° N zone the five warmest years are 1937, 1938, 1943, 1944 and 1953, and the five coldest years are 1964, 1965, 1966, 1968 and 1972. The average temperature difference between the warm and cold year groups is 1.6 °C for the high latitude zone, and 0.6 °C for the Northern Hemisphere as a whole, and the years themselves reflect the general warmth of the 1930s and 1940s and the subsequent cooling of the Northern Hemisphere. When the winters are compared (for the high latitude zone), the warm-year group is, on average, 1.8 °C

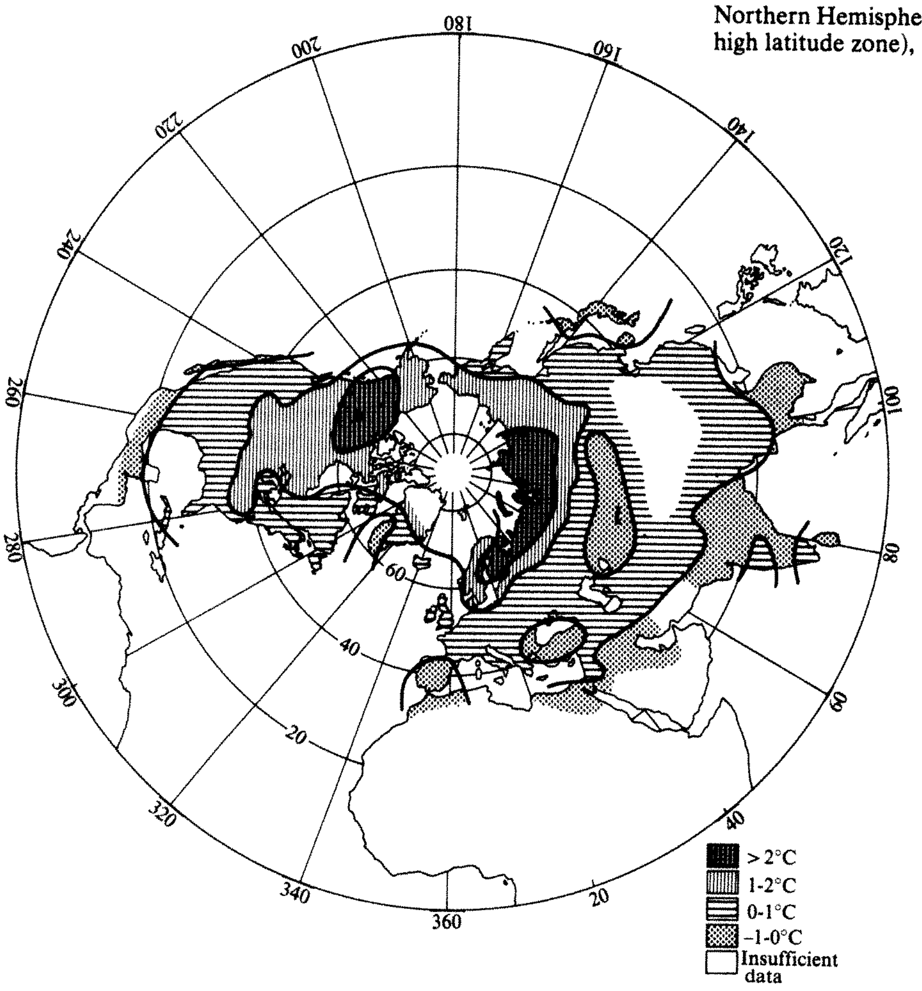

Fig. 1 Mean annual surface temperature changes from cold to warm years. The corresponding change in the hemispheric mean temperature is 0.6 °C. For reference, the expected change in global mean temperature due to a doubling of atmospheric CO_2 concentration is ~2 °C.

warmer than the cold-year group; for the summers the corresponding difference is 0.7 °C. This greater sensitivity in winter is in accord with expectations for the high-CO_2 world based on numerical modelling results[10]. All of these differences are statistically significant at the 5% level; the annual differences being significant at better than the 0.1% level. This is an important point since it justifies the use of the two groups as analogues of separate populations, even though they have been derived from a single population.

We now find the spatial patterns of temperature and precipitation differences between the cold- and warm-year groups. These patterns (Figs 1 and 2) provide a scenario for (but not a prediction of) the changes in climate which might accompany a CO_2-induced global warming. Since modelling results suggest that a doubling of CO_2 will warm the globe by 2 °C or more, the magnitude of CO_2-induced temperature changes may be somewhat larger than the differences shown in Fig. 1.

Figure 1 shows the spatial pattern of mean annual surface temperature differences between the cold- and warm-year groups. This map has been constructed from station rather than grid-point data, based on data from 219 stations. Maximum warming occurs in high latitudes and in continental interiors; up to more than five times the hemispheric mean increase in a region extending from Finland across the northernmost parts of Russia and Siberia to about 90° E. Not surprisingly, this area coincides with the region of greatest natural temperature variability. A large part of North America has positive temperature changes of two or more times the hemispheric mean, and this region extends westwards from Alaska, across northern Asia and into Scandinavia. In contrast, some regions show negative differences; Japan, much of India, an area including and adjacent to Turkey, the Iberian peninsula and adjacent North Africa, the south-west coast of the US, a region in central Asia and southwestern Greenland.

An examination of the surface pressure pattern differences between cold and warm years shows that the warm years have intensified high-latitude (50° N to 70° N) westerlies, greater cyclonic activity in the arctic and subarctic regions of the eastern hemisphere, and a westward displacement of the Siberian High in the winter half of the year. These features are consistent with the observed temperature differences. The shift in the Siberian High helps to explain the cooling zone in central Asia, which is largely an autumn and winter phenomenon.

Figure 1 only shows differences in mean annual temperature. Seasonal temperature changes are equally important and require further analysis. Most of the large differences which occur at high latitudes, although in the same sense for all seasons, are greatest in winter. In North America there is, in general, warming in all seasons: in the central region north of 40° N the warming is greatest in autumn, while for the rest of the continent (including Alaska) it is greatest in winter. The cooling zone around Turkey is due mainly to cooler springs: summers are generally slightly warmer. In India the cooling is greatest in summer and autumn; in winter and spring there are minor differences of both signs and a considerable degree of spatial variability. Finally, the cooling of the south-west coast of the US is a maximum in summer and the winters are actually warmer.

Figure 2 shows the distribution of precipitation changes from cold to warm years, based on data from 215 stations (most, but not all, being those used in Fig. 1). Precipitation patterns generally have greater small-scale spatial variability than temperature patterns, so the picture presented here is only a guide to broad-scale patterns. When averaged over the Northern Hemisphere we found that the warm-year groups showed a slight (statistically insignificant) increase in annual precipitation of between 1 and 2% compared with the cold-year group. Numerical modelling results indicate that an overall increase of around 5% might accompany a doubling of atmospheric CO_2[10].

The most important features of Fig. 2 are decreases in precipitation over much of the US, most of Europe and Russia (especially France, Spain and the Central Russian Plain) and over Japan, and increases in precipitation over India and the Middle East. For India, the increases vary from a few per cent in Bangladesh and along the eastern coast, to almost 100% in the north-west. Decreases in precipitation occur in southern India and in the north around New Delhi. In spite of these considerable variations, a clear spatial pattern is apparent, indicative of a more intense monsoon circulation in the warm years. Some of the features in Fig. 2 are the same as those which occurred during the Hypsithermal, the period used by Kellogg[8,9] as an analogue for a future high-CO_2 world. However, there are notable differences. Kellogg shows a wetter Europe and Russia and drier conditions over northwestern Canada, in contrast to our results. Some of these differences probably arise because Kellogg's reconstruction reflects summer precipitation, but the different boundary conditions which prevailed during the Hypsithermal must also be a significant factor.

The seasonal distribution of precipitation changes is important in determining impact. In particular, the consequences of a drier (and warmer) Europe and Russia deserve further consideration. The reductions in rainfall shown in Fig. 2 are not evenly distributed over the year. Largest reductions occur mainly in winter and autumn; but most regions (especially eastern Europe) also show reductions in summer precipitation. A few stations (Lyons, Madrid, Lisbon, Vienna) have greater summer rainfall in the warm years. In the UK annual precipitation changes are minor, except for southern England where precipitation is less in the warm years (spread fairly evenly over winter, spring and summer). For other parts of the UK, although annual changes are small, most places show reductions in spring rainfall and increases in summer rainfall. For the central and western parts of the USA, where there is a reduction in annual precipitation, increases in the winter and spring and decreases in the summer and autumn dominate the picture. Further north, in Canada, summer precipitation increases.

Changes in boundary conditions

The present results are based on climatic fluctuations which occur on the year-to-year time scale. Climate changes on many time scales (in fact, the effects of increased CO_2 are relatively slow), and there are important changes which have occurred on time scales too long to be noticeable in the record of the past 50 years. In general, these slow changes have been associated with larger changes in ocean and cryosphere boundary conditions than have been observed this century. Similarly, a large warming at high latitudes will probably lead to changes in Arctic sea ice extent, ocean current systems and sea surface temperatures which are outside the range of variations experienced during the past 50 years. These changes in the boundary conditions for the atmosphere require further discussion since they are central to the use of the past as an analogue and to the use of GCMs in modelling conditions in a warmer world.

It has been suggested that CO_2-induced warming may cause seasonal melting of the Arctic sea ice[8,16,27]. How much warming is required for this is uncertain. Manabe's simulation with four times the pre-industrial CO_2 level [10] showed the sea ice disappearing in summer, and this is commensurate with the work of Budyko[26] and Parkinson and Kellogg[27] which suggests that a summer temperature increase of 4–5 °C in the Arctic Basin would be required to melt the sea ice in summer. These results indicate that sea ice boundary conditions will probably not alter significantly beyond the extremes of the past 50 years at least until the early decades of the twenty-first century: although proposed alterations to the flows of major northward-flowing Russian rivers[28,29] might accelerate Arctic sea ice changes.

We have examined sea ice extent in our cold and warm year groups and found there to be notable differences. Two of the warm years (1937 and 1938) were remarkable for a major reduction in the summer ice along the north coast of Asia and increased outflow of ice from the Arctic in the East Greenland current. Further details are given by Koch[30].

Sea-surface temperature (SST) patterns form the other main atmospheric boundary condition. In GCM simulations, SSTs are

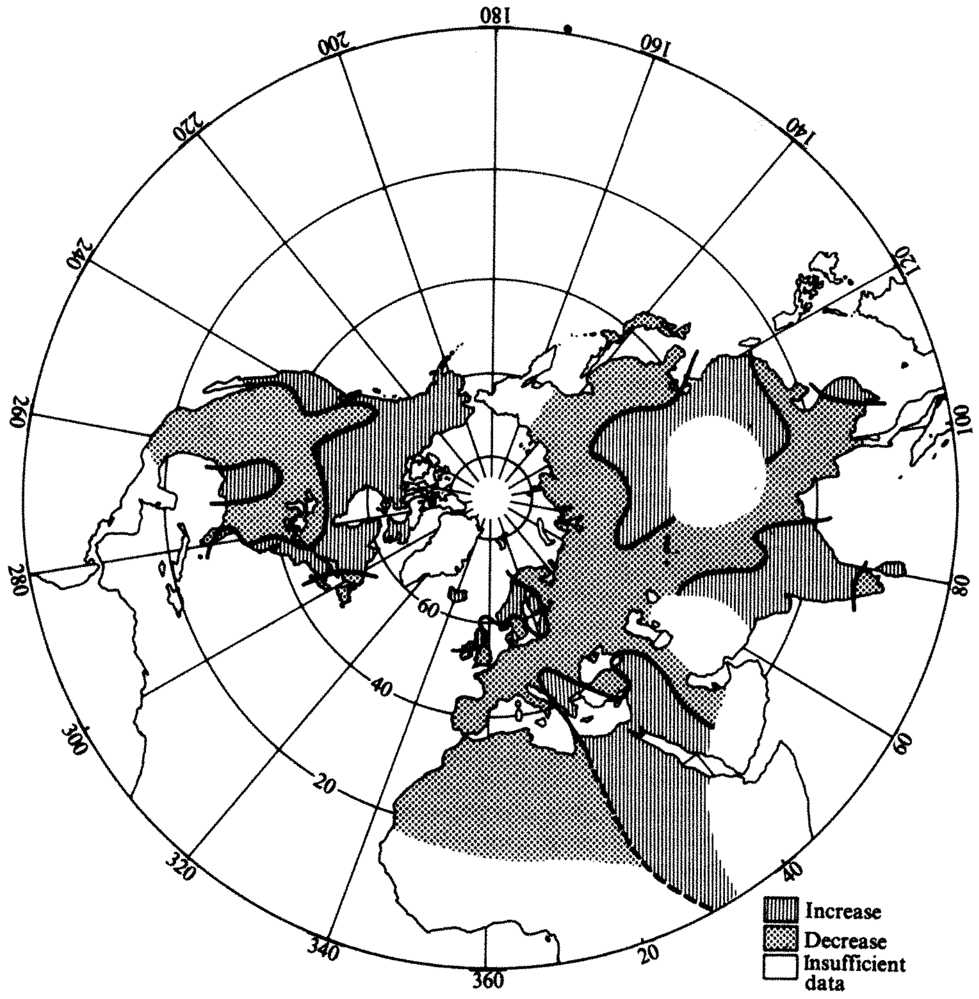

Fig. 2 Mean annual precipitation changes from cold to warm years.

either prescribed (in atmosphere-only models) or determined interactively (in coupled ocean–atmosphere models). Because of data deficiencies we have not been able to examine SST differences between our cold and warm year groups in any detail. We find however, a strong indication that SSTs over most of the North Pacific were cooler in the warm years. Ocean currents are also important, partly because of their relationship with SST patterns, but also because they are responsible for large amounts of latitudinal heat transport. No GCM with realistic ocean currents has been applied to the CO_2 problem. There are insufficient data available to study the details of changes this century, but we note that quite large changes in the Gulf Stream are thought to have occurred in earlier centuries[31].

Ocean current changes are linked to changes in atmospheric circulation patterns; in particular to changes in surface wind stress. Any significant wind stress reduction would be important because it would result, not only in a change in ocean current dynamics, but also in reduced mixing in the upper layers of the ocean and reduced upwelling, both of which would have profound effects on marine life through changing nutrient supply rates. Budyko and Vinnikov[32] have argued that the mean surface wind stress in a high-CO_2 world might be as little as 50% of today's value. In examining the mean surface wind stress (based on the geostrophic wind) in our warm and cold year groups we found the differences to be small, amounting to only a few per cent and contrasting with the prediction of Budyko and Vinnikov[32]. Although this result is reassuring, further examination of possible regional changes in wind stress is required.

Conclusions

We have used the word 'scenario' deliberately to distinguish our results from a 'prediction'. A scenario can only provide a guide to the patterns of climatic change which could occur accompanying a global warming. Our results are limited to relatively small changes in ocean and cryosphere boundary conditions, changes which might well be exceeded in the early decades of next century. Further, as the average hemispheric mean temperature differences between the warm and cold year groups is 0.6 °C, and because changes in regional climate may be non-linear with respect to global or hemispheric temperature change (especially when large changes in boundary conditions are involved), our scenario is most probably only representative until around the turn of the century. It would be dangerous to put any faith in extrapolations which involve hemispheric mean temperature shifts of more than 0.6 °C from the present.

Because the scenario presented here is based on mean conditions for two five-year groups, it is only representative of 5-year to decadal mean conditions. Superimposed on such conditions one must expect a 'normal' amount of year-to-year variability. Whether this variability will be less or more than the present is a controversial issue. Although a link between mean surface temperatures and mean variability may exist, the link is neither simple nor obvious, as van Loon and Williams[33] have shown. We can be sure that the climate will continue to vary considerably from year to year (just as the individual years in our warm and cold groups show noticeable differences), so even in a warm high-CO_2 world there will be regional extremes of warmth and cold, wetness and dryness which will differ markedly from 5 to 10-year mean patterns.

What would be the human (social, economic and political) impact of changes of the type presented here? We can only speculate on some of the possibilities—while noting that impact is at least as much related to the year-to-year variability of climate as it is to the mean or to changes in the mean. First, a substantial increase in the intensity of the Indian monsoon could have a significant impact. Although the impact of the monsoon in India is generally linked to its failure, increased and more violent precipitation can also have most detrimental effects (as witnessed by the flooding in 1979). Second, in some parts of the world (in particular in Europe and European Russia) temperature increases may occur in association with reductions in precipitation. These two factors will reinforce each other in increasing soil moisture deficits and so tend to have greater agricultural impact than either factor would alone. The climatic differences between warm and cold years which we have shown for Europe and European Russia appear to be of special importance as they indicate that conditions in a high-CO_2 world could be radically different from those given by Kellogg for the Hypsithermal.

We stress that the results presented here are little more than a guide to the type of climatic conditions that are possible in a high-CO_2 world. Should a global warming occur it will, however, certainly affect the weather patterns and climate regimes of the world and the effects will be different in different regions. These changes will, at the very least, result in shifts of agricultural zones, and some regions will be affected adversely, some favourably. Although some of the effects of a global warming (caused by CO_2 increases, or for any other reason) may well be beneficial[34], our scenario does show that detrimental effects could occur in some parts of the world, and our work highlights the need for more research into the interdisciplinary problems associated with climatic change which are focused by the current interest in the CO_2 issue.

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Oxygen and strontium isotope relationships in the British late Caledonian granites

Russell S. Harmon & Alex N. Halliday

Isotope Geology Unit, Scottish Universities Research and Reactor Centre, East Kilbride, UK

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Oxygen and strontium isotope data for 25 late Caledonian granite plutons emplaced between ~435 and 390 Myr indicate that the parent magmas were hybrids, derived from partial fusion of mantle-like material and varying proportions of a crustal component.

COMBINED stable and radiogenic isotopic studies can provide important information concerning both the origin of granite magmas and the post-emplacement history of volcanic and plutonic igneous rocks[1,2]. In this article we present the results of a combined O–Sr reconnaissance isotopic study of twenty-five ~400-Myr-old Caledonian granitic plutons from Scotland and northern England and assess the extent to which crustal anatexis and/or assimilation was important in the generation and subsequent evolution of the parent magmas.

The Caledonian fold belt is thought to have formed as a result of the closure of the 'Iapetus' ocean basin during Lower Palaeozoic times[3]. Granitic magmas were intruded into Precambrian and Lower Palaeozoic country rocks between approximately 640 and 390 Myr (refs 4, 5). Most important spatially and volumetrically are the late Caledonian granites, the 'Newer' and 'Last' granites of Read[6]. These post-tectonic, largely undeformed granites include those emplaced by active injection into country rock with concordant migmatisation and veining (forceful 'Newer' granites) and those emplaced in ring complexes associated with tensional faulting and cauldron subsidence (passive 'Last' granites). Read[6] separates these two classes in time, arguing that deposition of the Lower Old Red Sandstone occurred between emplacement of the forceful 'Newer' and passive 'Last' granites. Apart from those of the north-east Grampian region[4] only a few reliable radiometric ages for the forceful 'Newer Granites' have been published (for example, Strontian, 435 ± 10 Myr)[7] and the oldest reasonably reliable published age for a passive cauldron subsidence granite is that for Lochnagar (415 ± 5 Myr)[5]. However, the temporal classification of Read[6] cannot be applied throughout the British Isles. In Donegal, for example, forceful emplacement (the Main Donegal granite) post-dates passive emplacement (Rosses

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Table 1 Rb–Sr analyses (new) for four Grampian Highland granitoids

Pluton*	Rb (p.p.m.)	Sr (p.p.m.)	Rb/Sr (weight)	^{87}Rb/^{86}Sr (atomic)	^{87}Sr/^{86}Sr (atomic)	(^{87}Sr/^{86}Sr)$_t$†
Ballachulish-I	65.72	1568	0.04193	0.1213	0.70495	0.70424±4
Ballachulish-II	85.88	1203	0.07141	0.2066	0.70553	0.70433±6
Ben Nevis	101.1	734.2	0.1377	0.3982	0.70662	0.70430±5
Moor of Rannoch	62.03	1581	0.03923	0.1135	0.70548	0.70482±7
Garabal Hill	94.40	638.2	0.1479	0.4280	0.70988	0.70741±7

All analyses utilised isotope dilution and an MM30B mass spectrometer. Uncertainties (2σ) are ±0.7% for ^{87}Rb/^{86}Sr and $<\pm 0.01\%$ for ^{87}Sr/^{86}Sr. NBS 987 gave a value of 0.71020±3 (2σ for two analyses) at the time of these measurements.

* Sample locations and ages are given in Table 2.

† Combines estimated uncertainties for (^{87}Sr/^{86}Sr) with (^{87}Rb/^{86}Sr)($e^{\lambda t}-1$) quadratically; uncertainty in t (the estimated age) not included; $\lambda^{87}\text{Rb} = 1.42\times10^{-11}$ yr^{-1}.

Complex) with all granites emplaced close to 410 Myr (ref. 8 and A. N. H., M. Aftalion and B. E. Leake, unpublished). Similarly, the Fleet pluton of the Southern Uplands in Scotland was emplaced forcefully at 392±2 Myr (ref. 9).

In general, the late Caledonian granites have the calc-alkaline characteristics typical of plutonic rocks emplaced at destructive plate margins[9–11]. The 25 plutons sampled in this study comprise a majority of the late Caledonian granites but do not include the ~460-Myr-old granites studied by Pankhurst[4]. Many of the plutons are composite and zoned. The samples analysed, broadly described as 'granitic', range in composition from diorite and tonalite, through granodiorite, to peraluminous granite with chemical variation continuous among the various rock types. Petrographically and chemically the granitoids span the range from 'sedimentary' to 'igneous' type as defined by Chappell and White[12] with no discernible geographical pattern to their distribution. Recent zircon U–Pb, Rb–Sr, and common Pb reconnaissance studies[5,7,13] have revealed a general pattern of inherited zircon U–Pb memory north of the Highland Boundary Fault, generally low initial Sr isotope ratios (<0.709), and a tendency for Pb to be increasingly radiogenic southwards across the region.

Isotopic ratio determinations

Oxygen was extracted from whole rock powders by reaction with BrF_5 and converted to CO_2 as described by Clayton and Mayeda[14]. ^{18}O/^{16}O ratios, determined on the CO_2 gas using an AWRE Aldermaston isotope ratio mass spectrometer, are reported here in the 'δ' notation relative to standard mean ocean water (SMOW) as defined by the Snowbird Quartz (+16.20‰) and African Glass Sand (+9.60‰) standards. Overall precision is 0.1–0.2‰. The Sr isotope ratios presented here are chiefly those reported by Halliday *et al.*[5,9] but include new data (Table 1) for four southwestern Grampian Highlands granites (Ben Nevis, Ballachulish, Moor of Rannoch, Garabal Hill). (^{87}Sr/^{86}Sr)$_i$ ratios were calculated from (^{87}Sr/^{86}Sr) ratio measurements corrected for ^{87}Rb decay using independent K–Ar, Rb–Sr and U–Pb ages or from Rb/Sr isochron regression. Samples for which the ages are not accurately known (such as Cluanies) have such low Rb/Sr ratios that the calculated (^{87}Sr/^{86}Sr)$_t$ ratios are very close to initial ratios.

The O and Sr isotopic data for this reconnaissance study of the ~400 Myr Caledonian granitoids are presented in Table 2 and, averaged for each pluton, are plotted geographically in Fig. 1. No regional distribution pattern is apparent for either δ^{18}O values or (^{87}Sr/^{86}Sr)$_i$ ratios. An area of low δ^{18}O and (^{87}Sr/^{86}Sr)$_i$ is present around Glencoe (Ben Nevis–Ballachulish–Moor of Rannoch) and there is a general tendency for the granites of the southwestern Grampian Highlands and Northern Highlands to have slightly lower δ^{18}O values than those of the Southern Uplands and northeastern Grampian and Northern Highlands. Furthermore, no correlation is observed between either δ^{18}O or (^{87}Sr/^{86}Sr)$_i$ with distance from the 'Iapetus' suture in the Southern Uplands as proposed by Brown and Hennessy[15].

Magmas derived from the mantle or a primitive (basic or ultrabasic) mantle-derived source have a characteristic isotopic composition: $\delta^{18}\text{O} = 6.0\pm0.5$‰ (refs. 16, 17) and (^{87}Sr/^{86}Sr)$_i$ = 0.703±0.001 (ref. 18). Fractional crystallisation of a magma may cause slight ^{18}O enrichment, but should not affect the ^{87}Sr/^{86}Sr ratio. However, crustal processes such as weathering, hydrothermal alteration, and metamorphism can produce substantial changes in oxygen isotopic composition. Additionally, the crust has developed a high ^{87}Sr/^{86}Sr ratio because of the time-dependent effect of its generally higher Rb/Sr ratios. As a result, the crust is enriched in both ^{18}O and ^{87}Sr relative to the mantle and therefore it should be possible to recognise granites produced by crustal melting from those generated in the mantle or lower crust on the basis of their O and Sr isotopic composition. The degree to which a crystallised granite will reflect the

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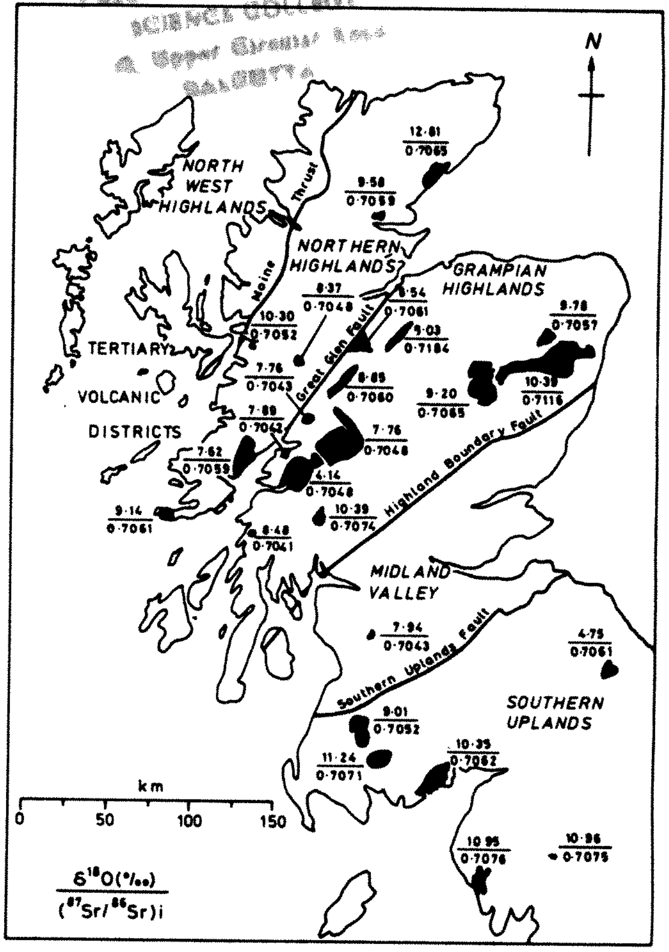

Fig. 1 Location map showing the physiographical provinces and major tectonic features of northern England and Scotland, the geographical distribution of the 'granites' sampled in this study, and the average δ^{18}O values and initial Sr isotope ratios for each pluton. The proposed 'Iapetus' suture line is considered to trend NE–SW across the Southern Uplands in the vicinity of the Solway Firth.

exact oxygen isotopic composition of the source(s) will depend on several factors, among them the temperature of melting, the degree of partial melting, the degree of volatile loss from the melt, the amount of fractional crystallisation, and the extent of post-emplacement alteration. For example, the extreme ^{18}O enrichment observed in the Tuscan Igneous Province (δ^{18}O = 11 to 17‰)[19] and the high initial ^{87}Sr/^{86}Sr ratios characteristic of the ~460-Myr-old Caledonian granites (>0.71)[4] can only be explained in terms of fusion of large amounts of crustal material. On the other hand, ^{18}O depletion can result from post-emplacement interaction with meteoric fluids or by partial fusion of materials previously affected by such a process. The ^{18}O depletion of 5 to 10‰ observed within 2 to 3 stock diameters of most intrusive centres within the Scottish Tertiary Volcanic District[20,21] is typical of such post-emplacement subsolidus exchange.

In Fig. 2 the O and Sr isotopic data for the 25 plutons are shown in histogram form. The samples analysed were, in part, those previously studied by Pidgeon and Aftalion[7] and Halliday *et al.*[5,9] for their U–Pb zircon and Rb–Sr isotopic systematics and thus do not represent a statistically valid sampling of the 'Newer' granites because, in certain instances, multiple samples were taken from individual plutons and, in others, from single outcrops (Table 2). Variations in δ^{18}O and (^{87}Sr/^{86}Sr) are observed in many of these plutons so that it is difficult to represent the isotopic composition of an intrusion by a single sample[9]. Therefore, in cases for which more than one sample was analysed from a single location (such as Bonar Bridge) or for which the initial Sr isotope ratio was derived from a whole-rock isochron regression on a suite of samples from a particular pluton (such as Lochnagar), only a single, averaged δ^{18}O and (^{87}Sr/^{86}Sr) value for that pluton has been plotted in Fig. 2. Otherwise, where more than one outcrop of a particular pluton was sampled (as for Doon), each data point is shown.

The large range in both δ^{18}O values (4.1–14.4‰) and (^{87}Sr/^{86}Sr)$_i$ ratios (0.7037–0.7184) is apparent. No correlation is observed between either isotopic parameter and emplacement age or intrusion style, except that the two plutons with extremely high (^{87}Sr/^{86}Sr)$_{400}$ ratios (Findhorn and Aberdeen) may be significantly older than the other granites sampled. Although the ages for these two plutons are uncertain, their Rb/Sr ratios are sufficiently low that this does not nullify their distinctively high initial Sr isotopic ratios. Substantial within-pluton variation in both δ^{18}O and (^{87}Sr/^{86}Sr)$_i$ coincident with compositional trends are observed for those composite plutons studied in detail (Table 1).

No significant differences are observed for either isotopic parameter between the forceful and permitted granites sampled except that the two samples with anomolously low δ^{18}O values Cheviot and Ben Cruachan, are both high-level probably sub volcanic, 'Last' granites. The ^{87}Sr/^{86}Sr initial ratios for Moor of Rannoch and Ballachulish (forceful 'Newer' granites) are effectively identical to those of Ben Nevis and Ben Cruachan (passive 'Last' granites). Thus, the recent suggestion by Brown and Locke[22] on the basis of recent trace element and geophysical studies[23–25] that the 'Older' granites, as defined by Read[6], and the forceful 'Newer' granites are genetically related and largely derived from continental crust whereas the passive 'Last' granites were derived largely from the mantle is incompatible with the isotopic data presented here and the important isotopic work of Pankhurst[26] on the Strontian and Foyers granites.

The generally low (^{87}Sr/^{86}Sr)$_i$ ratios and extremely variable ^{18}O/^{16}O ratios observed preclude an origin of the forceful 'Newer' granites simply by partial fusion of highly evolved crustal materials as detailed by Pankhurst[4] and Bell[27] for the 'Older' granites and ~460-Myr-old Aberdeenshire granites. Rather, we consider the 'Newer' and 'Last' granites to comprise a distinct magmatic group, as first proposed by Read[6], which were chiefly formed between ~435 Myr and 390 Myr and that the observed field differences are related to a time-variable local tectonic setting rather than being a genetic characteristic of the granites themselves.

Of particular interest are the two samples which show extremely low δ^{18}O values of <5‰ (Ben Cruachan and Cheviot). These two ^{18}O/^{16}O ratios which represent the first recorded occurrence of pre-Tertiary low-^{18}O granites in the British Isles, are clearly anomalous within the context of the other Late Caledonian granites sampled. Their oxygen isotopic composition can only be explained in terms of either post-emplacement exchange between the granite plutons and ^{18}O-depleted meteoritic groundwater or a derivation from partial fusion of pre-existing low-^{18}O crustal rocks. (^{87}Sr/^{86}Sr)$_i$ ratios for the two samples show no major enrichment with radiogenic Sr as would be expected if they had originated from a crustal source (Ben Cruachan = 0.7048; Cheviot = 0.7061). Pankhurst *et al.*[28] have shown that Sr isotope ratios are not necessarily affected during hydrothermal interaction which has resulted in substantial ^{18}O depletion. Although Forester and Taylor[20,21] have documented that the southern porphyritic epigranite of the Tertiary Red Hills complex, Skye was intruded as a low-^{18}O magma derived from the partial melting of altered metasediments, we favour a post-emplacement cause because both are high-level, subvolcanic centres where the effects of such hydrothermal processes are most likely to be present.

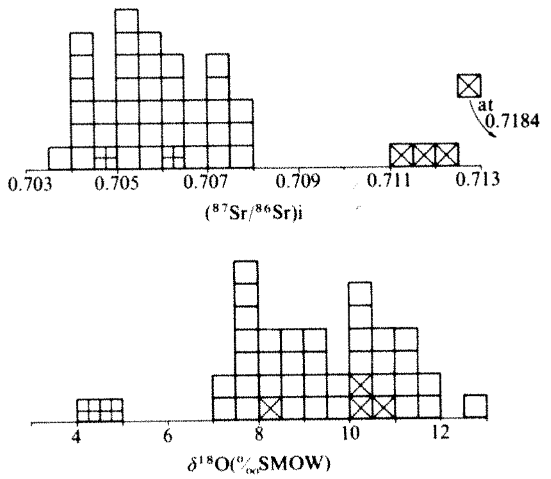

Fig. 2 Histograms of δ^{18}O and (^{87}Sr/^{86}Sr)$_i$ for the 'granites' sampled. The two low-^{18}O samples (Cheviot and Ben Cruachan) are indicated by vertical crosses and the extremely ^{87}Sr-enriched samples of uncertain age (Findhorn and Aberdeen) by diagonal crosses.

It is also seen from Fig. 2 that approximately one quarter of the granites sampled have δ^{18}O values which lie within Taylor's[16] I and H$_{\rm I}$ groups for plutonic igneous rocks. Although the oxygen isotopic composition of these granites is compatible with a mantle or primitive mantle-derived source, allowing for a 1 to 1.5‰ enrichment in ^{18}O during fractional crystallisation, their initial strontium isotope ratios require that the magmas had to some extent interacted with continental crust during their evolution. Previous O-, Sr-, and Pb-isotopic studies have recognised the involvement of crustal materials in the production of granitic magmas[4,7,16,29–32]. However, the exact nature and degree of this involvement remains unclear. Chappell and White[12] and O'Neil and Chappell[33] have pointed out that it may be possible to recognise granitic rocks derived primarily from metasedimentary crustal sources (S type) and those with primitive igneous origins (I type) on the basis of their petrologic, chemical and isotopic compositions. As regards the Caledonian granites, Pankhurst[26] has pointed out that there is a clear distinction between 'Older' and 'Newer' granites. The 'Older' suite is comprised of small, two-mica granites which have low Ca and Na/K with high (^{87}Sr/^{86}Sr)$_i$, a predominance of biotite over hornblende, and true granitic compositions; all characteristics of an S type affinity. By contrast, the 'Newer' and 'Last' granites are generally large, complex and often composite plutons ranging from diorite to granite in composition, with hornblende, and high Ca and Na/K with low $(^{87}Sr/^{86}Sr)_i$. This would suggest that these late Caledonian granites should be predominantly of I type, but such a categorical classification is not supported by our O and Sr isotopic data.

Relationship between oxygen and strontium isotope ratios

Figure 3 is a plot of $\delta^{18}O$ against $(^{87}Sr/^{86}Sr)_i$ for the 25 granites analysed. The two low-^{18}O samples (Ben Cruachan and Cheviot) and the two extremely ^{87}Sr-enriched samples (Findhorn and Aberdeen) have been omitted. The compositional trends previously described are clearly illustrated. Both $\delta^{18}O$ and $(^{87}Sr/^{86}Sr)_i$ tend to increase sympathetically with the progression from more mafic to more felsic compositions. The $\delta^{18}O$ values for this group of late Caledonian granites as a whole appear to form a continuous distribution across the S–I boundary of ~10‰ suggested by O'Neil and Chappell[33] and O'Neil *et al.*[34] for the Australian Palaeozoic granites with no tendency for the forceful 'Newer' granites to be more S type in nature than the passive 'Last' granites.

The most important feature of Fig. 3 is the positive correlation between $\delta^{18}O$ and $(^{87}Sr/^{86}Sr)_i$ which is statistically significant ($r^2 = 0.75$) at the 99% confidence level. That this trend is parallel to that observed by Taylor and Silver[2] for the Peninsular Ranges batholith of California and Baja would strongly imply that a fundamental petrogenetic process must be responsible for the sympathetic O and Sr isotope variation observed. Differential partial melting of a single source or derivation from a single partial melt at each site are effectively ruled out by the large oxygen isotope variations observed within and between individual plutons. Incidentally, a major difference between the British Caledonides and California/Baja is the striking geographical effects observed in the isotopic systematics of the latter[2], which is probably due to its simpler geology setting and history.

It is also noteworthy that positive correlations of $(^{87}Sr/^{86}Sr)_i$ with Rb/Sr are observed for the late Caledonian granites which is also suggestive of magma mixing. Halliday *et al.*[5] have considered possible sources for an upper crustal component: Lewisian, Moinian and Dalradian metamorphic rocks and Lower Palaeozoic sediments. Whilst all fulfill the requirements imposed by the granite U–Pb zircon systematics, the Southern Uplands Lower Palaeozoic sediments are the only abundant upper crustal material that could provide a melt derivative of the required Sr isotopic composition. An oxygen isotopic study of these sediments[9], the result of which is shown in Fig. 3, indicates that such material would be suitable upper crustal end member composition for the magma mixing situation we envisage for the late Caledonian granites. For example, the Shap and Eskdale

Table 2 Oxygen and strontium isotope data for 25 Caledonian granitoids

Pluton	Age (10^6 yr BP)	Locality (Natn. grid. ref.)	Rock type	$\delta^{18}O$ (‰ SMOW)	$(^{87}Sr/^{86}Sr)_i$
Helmsdale	420	ND 028158	lg	12.8	0.7065
Bonar Bridge	400	NH 663913	lg	9.5	0.7059
Aberdeen*	?	NJ 866135		10.1	0.7119
		NJ 906106		10.5	0.7120
		NJ 914077		10.5	0.7114
Hill of Fare	413	NJ 711006	lg	9.2	0.7059
		NJ 673011		9.4	
		NJ 737033	lg	11.06/11.08	
		NJ 728051	lg	9.4	
				9.8	
Findhorn	?	NH 726260		8.0	0.7184
Foyers	400	NH 548204	gd	8.5	0.7061
Ratagan	400	NG 901198	gd	10.3	0.7052
Cluanies	417	NH 175104	gd	8.4	0.7048
Strath Ossian	400	NM 371809	gd	8.8	0.7060
Lochnagar	415	NO 340900	g	9.4	0.7065
		NO 253863	g	9.6	
		NO 260805	g	8.7	
		NO 268793	g	9.1	
				9.2	
Strontian	435	NM 821608	gd	7.1	0.7052
		NM 809533	gd	7.3	0.7054
		NM 780650	g	8.5	0.7070
Ben Nevis	410	NM 115716	g	7.8	0.7043
Ballachulish (I)	410	NN 025594	gd	7.8	0.7042
(II)		NN 021589	gd	8.0	0.7043
Moor of Rannoch	410	NN 465571	gd	7.8	0.7048
Ben Cruachan	410	NN 039333	gd	4.1	0.7048
Ross of Mull	414	NM 303230	g	9.1	0.7061
Garabal Hill	406	NS 306188	gd	10.4	0.7074
Kilmelford	427		d	9.4	0.7044
			d	7.5	0.7038
Distinkhorn	390	NS 599350	d	7.9	0.7043
Cheviot	390	NT 917164	g	4.71/4.78	0.7061
Doon	408	NX 467771	d	8.3	0.7042
		NX 477787	d	7.8	0.7050
		NX 449784	gd	8.3	0.7052
		NX 458818	g	10.2	0.7052
		NX 455887	g	10.3	0.7059
Criffell	397	NX 950690	gd	8.5	0.7052
		NX 870570	gd	9.0	0.7056
		NX 902606	g	10.6	0.7059
		NX 900659	g	11.8	0.7069
		NX 910660	g	11.8	0.7073
Cairnsmore of Fleet	392	NX 548751	g	11.2	0.7062
		NX 568727	g	11.2	0.7072
		NX 560720	g	11.3	0.7074
		NX 570710	g	11.3	0.7076
Shap	394	NY 555085	g	11.0	0.7075
Eskdale	429	SD 113944	g	10.9	0.7076

* Strontium isotope initial ratios calculated assuming 400 Myr age, but may be older.

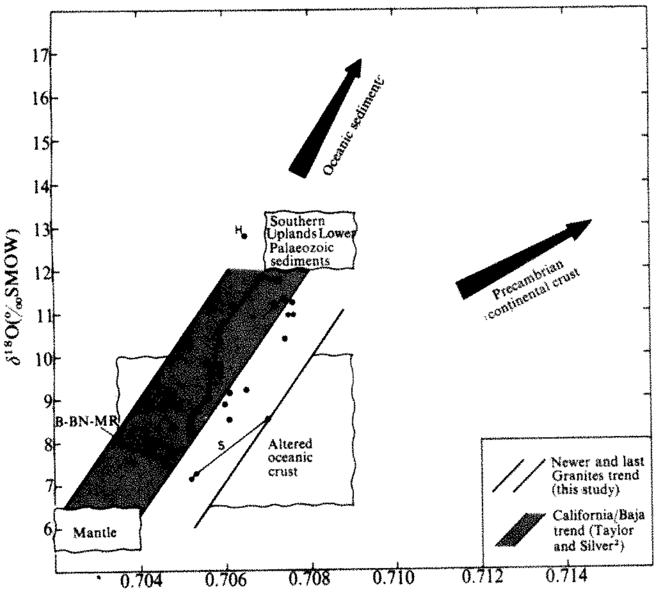

Fig. 3 $\delta^{18}O$–$(^{87}Sr/^{86}Sr)_i$ relationships for the 'granites' sampled. Not shown are the data for Cheviot, Ben Cruachan, Findhorn and Aberdeen. Isotopic variations corresponding to compositional trends are shown for the 'forceful' granites—Strontian and Criffell. The point indicated by 'H' is the Helmsdale granite and the B–BN–NR field is that for the Ballachulish, Ben Nevis and Moor of Rannoch granites. The shaded trend within the 'Newer Granites' trend is that observed by Taylor and Silver for the Peninsular Ranges batholith in California and Baja. For reference the fields of 'Mantle', 'Altered Oceanic Crust' and 'Southern Uplands Lower Palaeozoic Sediments' are shown together with postulated mixing trends between 'Mantle' and 'Precambrian Continental Crust' and 'Mantle' and 'Oceanic Sediments'.

granites of the Lake District and Fleet of the Southern Uplands plot with high $\delta^{18}O$ and $(^{87}Sr/^{86}Sr)_i$ and lie close to the field of Southern Uplands Lower Palaeozoic sediments shown in Fig. 3. The 'sedimentary' nature of these granites is clear and the conclusion must be drawn that they were largely derived from the geosynclinal metasedimentary wedge in which they occur. However, such sediments do not outcrop north of the Highland Boundary Fault, and even if underthrust during subduction, neither these geosynclinal sediments nor a severely altered oceanic basaltic slab could provide the inherited U–Pb component observed in zircons for granites north of the fault[5,9]. Some clues as to the nature of the crustal component have been provided by recent Pb isotope studies[13] from which it is apparent that a comparatively non-radiogenic Pb component is present in some of the Caledonian granites of the Northern Highlands (for example, Foyers and Ross of Mull). This evidence strongly suggests at least partial derivation of the Northern granites from an old, U-depleted crustal source such as Lewisian granulites. Nevertheless, both the Pb isotope data and the difficulty of extracting large volumes of 'granitic' magmas from granulites further support our contention that mantle or 'new' basic lower crust was also involved in the production of the late Caledonian granites. The large variations in both $^{18}O/^{16}O$ and $(^{87}Sr/^{86}Sr)_i$ ratios observed within single plutons (Fig. 3) together with the absence of U–Pb memory in the zircons of the Strontian tonalite contrasted with a marked inherited U–Pb component in the Strontian biotite granite[5,7] endorse this view. Pankhurst[26] has suggested on the basis of trace element and isotopic data that basaltic lower crust is a more likely candidate than mantle peridotite for the low-$^{87}Sr/^{86}Sr$ end member.

Discussion

It thus seems that each of the late Caledonian granites we have studied was derived from a variable mixture of mantle and/or basic lower crust and more isotopically evolved continental material analogous to the local upper crust. Further speculation is difficult because at present there are no oxygen isotope data on Precambrian crustal rocks of Britain other than the unpublished work of Kay[35]. The high $^{87}Sr/^{86}Sr$ ratios of all major Precambrian rock types (Lewisian, Moinian, Dalradian)[5] suggests that such a crustal melt would plot in Fig. 3 with a higher $^{87}Sr/^{86}Sr$ initial ratio for a given $\delta^{18}O$ value than the Southern Uplands Lower Palaeozoic sediments and thus not lie on the required mixing line to produce the general $\delta^{18}O$–$(^{87}Sr/^{86}Sr)_i$ trend observed. However, the partial melting of such ^{87}Sr-enriched Precambrian rocks may be locally important. This would provide an explanation for the tendency of the Strontian data to plot with lower slope than that of Criffell (Fig. 3). It is almost certain that more detailed studies of individual intrusions, such as that recently completed for the three Southern Uplands granites Doon, Criffell and Fleet[9], will reveal that the overall trend observed in Fig. 3 is comprised of a suite of correlations within the observed limits which relate to the local geological environment.

The model we envisage to explain the isotopic variations observed for the late Caledonian granites involves progressive upward melting from the mantle such that the first magma emplaced is derived from the most primitive source. The rise of granodioritic/tonalitic magma results in the transfer of heat to the surrounding crust sufficient to mobilise acidic melts at high levels in the crust. In some instances successive emplacement of more felsic magmas occurred in discrete pulses after hybridisation, whilst in others the magma pulses became progressively enriched in the crustal component. Simple modelling in a two-component system suggests that between 30 and 80 wt% of crustal contamination is required to account for the observed O and Sr isotopic variations.

This work is a continuation of a large-scale isotopic reconnaissance study of British granites begun by R. T. Pidgeon. We thank Professor H. W. Wilson for his support, J. Borthwick, J. Hutchinson and J. Jocelyn for technical assistance in the laboratory, and M. L. Coleman, S. M. F. Sheppard, W. E. Stephens, L. T. Silver, H. P. Taylor, P. E. Brown, C. Graham, I. Munro and A. Rickard for samples and discussion. The Isotope Geology Unit at SURRC is supported by a grant from NERC. Manuscript typed by Mrs A. McCartney.

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E. coli recA protein-directed cleavage of phage λ repressor requires polynucleotide

Nancy L. Craig & Jeffrey W. Roberts

Department of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, New York 14853

The recA protein mediates both genetic recombination and several cellular responses to DNA damage, including the induction of temperate bacteriophage. Induction of phage λ results from proteolytic cleavage of λ repressor directed by recA protein. We show here that this cleavage reaction requires both polynucleotide and ATP. We suggest that a stoichiometric complex of recA protein and DNA is active both to destroy repressors by proteolytic cleavage and to initiate pairing of this DNA to its homologous sequence in a DNA duplex ('strand invasion').

THE product of the *recA* gene of *Escherichia coli* appears to have two distinct roles; it is required for all pathways of general genetic recombination[1] and it regulates the expression of several important cellular processes, sometimes called the SOS functions, which are induced when cellular DNA is damaged or its replication is obstructed[2,3]. These processes include the development of temperate bacteriophage, the expression of colicinogenic plasmids[4], an inhibition of cell division that leads to filamentation, and an error-prone DNA repair capacity that is an important source of radiation and chemical mutagenesis. Furthermore, recA protein regulates its own expression: its rate of synthesis is greatly increased by inducing treatments, but this amplification requires *recA* function[5-7].

How does recA protein mediate these different processes? We suggest that it has two separate biochemical activities, which are related by the interaction of recA protein with DNA that we describe in this article. Others have shown that purified recA protein catalyses the pairing of homologous DNA strands, an activity that accounts for its direct role in genetic recombination[8-11]. We have shown that recA protein inactivates the repressors of phages λ and P22 *in vitro* by proteolytic cleavage, a finding that explains the role of *recA* function in phage induction[12-14]. This activity of recA protein to inactivate or modify proteins by proteolytic cleavage may underlie its regulatory function in the expression of other *recA*-dependent processes as well. One specific regulatory role of the proteolytic activity may be to promote the amplification of recA protein itself: the *lexA* gene product, thought to be repressor of the *recA* gene[15], could be inactivated by recA protein present at a low basal level before induction, thus causing depression of the *recA* gene[5-7].

Table 1 Rate of recA protein dependent cleavage of λ repressor in the presence of various polynucleotides

Polynucleotide	Polynucleotide concentration (μg ml^{-1})	Rate of cleavage (ng per min per μg recA protein) Low salt (0.06 M)	High salt (0.18 M)
1. None	—	<0.1	<0.1
2. Denatured λ DNA	1.5	38	12
3. Native plasmid DNA	22	7.2	0.3
4. poly(rA)	1.0	50	19
5. poly(dT)	1.5	43	10
6. $(dA)_{16}$	6.0	25	4.0
7. $(dT)_9$	20	25	4.9

Reaction mixtures of 20 μl were prepared as described in Fig. 1, using 1 mM ATP-γS, 2 mM $MgCl_2$, 3.4 μg repressor, 0.6 μg recA protein, polynucleotide as indicated, and salt as indicated. 'Low salt' was 0.015 M NaCl and 0.045 M KCl; 'high salt' was 0.135 M NaCl and 0.045 M KCl. After incubation for 45 min at 37 °C (low salt reaction) or 2 h at 37 °C (high salt reaction) cleavage was determined as described in Fig. 3. Homopolymers and oligonucleotides were obtained from P–L Biochemicals. Plasmid DNA was a derivative of pMB9, made linear by digestion with *Eco*RI endonuclease followed by phenol extraction.

We show here that the activity of purified recA protein to direct cleavage of phage λ repressor requires both polynucleotide and ATP. recA protein also associates with single-stranded DNA in the set of ATP-depenendent reactions that lead to pairing of this DNA to its homologous sequence in a DNA duplex ('strand invasion')[8,10,11]. We suggest that in the presence of ATP, recA protein binds in a stoichiometric complex with single-stranded DNA, and is thereby activated both to attack repressors and to engage in DNA strand pairing.

Repressor cleavage depends on ATP and polynucleotide

We have reported that purified recA protein promotes the specific proteolytic cleavage of purified λ repressor into two fragments, in a reaction that requires ATP and small molecules[12,13]. The requirement for polynucleotide originally was obscured by the presence of a contaminating polynucleotide synthetic activity in recA protein prepared as we previously described. Because this activity is inhibited by inorganic phosphate and because it sediments at about 9S (data not shown), it is probably polynucleotide phosphorylase, which utilises ADP derived from ATP to synthesise polyriboadenylic acid[16]. The experiment of Fig. 1 shows that λ repressor cleavage directed by highly purified recA protein is dependent on both ATP and polynucleotide, here denatured λ DNA. The ATP analogue ATP-γS[20] substitutes effectively for ATP and supports a several-fold higher rate of cleavage than does ATP. For this and all experiments reported here we used recA protein prepared from cells carrying the *recA* mutation tif^- (ref. 15), although we obtained qualitatively similar results using recA protein from tif^+ cells.

A variety of single-stranded polynucleotides support the cleavage of repressor by recA protein; native DNA is much less effective, particularly in reactions at higher salt concentrations (Table 1). Other polynucleotides that are active include denatured DNA restriction fragments several hundred nucleotides long, single-stranded DNA of phage G4, poly (rC) and poly(U) (data not shown). We also have detected cleavage in the presence of oligonucleotides as short as six nucleotides in the ATP-γS dependent reaction (data not shown). The finding that poly(rA) stimulates cleavage supports our suggestion that less purified *recA* fractions lack the polynucleotide requirement because poly(rA) is synthesised during the cleavage reaction. Because homopolymers are active, the formation of double-stranded polynucleotide[9] is not essential to the reaction.

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The rate of repressor cleavage in the ATP-γS stimulated reaction is approximately constant for several hours (Fig. 2). In optimal conditions (Table 1) the rate of repressor cleavage is 40–50 ng repressor per min per μg recA protein; ATP provides about one-fourth this rate. Thus recA protein promotes cleavage of an equimolar amount of repressor in about 15 min in these conditions. Both recA protein and represssor are present at concentrations that limit the rate of this reaction[14]. From the experiment of Fig. 3, track *a*, it can be seen that recA protein functions catalytically: 0.9 μg recA protein directed cleavage of 6 μg repressor, representing a turnover of 10 repressor monomers (molecular weight (MW) 26,000) per recA monomer (of MW 40,000). Figure 3, track *c* demonstrates that essentially all of the input repressor is sensitive to recA protein directed cleavage.

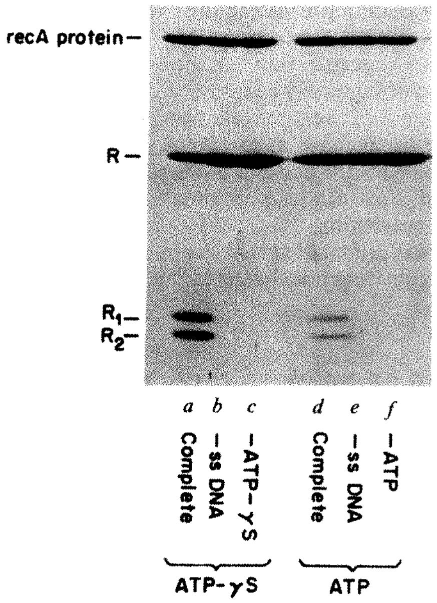

Fig. 1 Dependence of the recA protein directed cleavage of λ repressor on polynucleotide and ATP or ATP-γS. Reaction mixtures of 30 μl contained 13 mM Tris HCl *p*H 7.5, 1 mM potassium phosphate, 0.55 mM EDTA, 0.4 mM $CaCl_2$, 2.4 mM dithiothreitol, 0.145 M NaCl, 0.015 M KCl, 5% (w/v) sucrose, 4% (v/v) glycerol, 0.9 μg recA protein, 3.2 μg λ repressor, and 1.3 μg ml^{-1} heat-denatured λ DNA (unless omitted). Reactions *a–c* contained in addition 2 mM $MgCl_2$ and 1 mM ATP-γS (unless omitted); reactions *d–f* contained in addition 10 mM $MgCl_2$ and 5 mM ATP (unless omitted). Track *a*, complete reaction (ATP-γS); track *b*, DNA omitted; track *c*, ATP-γS omitted; track *d*, complete reaction (ATP); track *e*, DNA omitted; track *f*, ATP omitted. After incubation for 60 min at 37 °C reaction mixtures were analysed by SDS-gel electrophoresis[12] and staining with Coomassie brilliant blue. Bands corresponding to recA protein, λ repressor, and both fragments derived from λ repressor are indicated[12]. *tif*⁻-recA protein was prepared from *E. coli* DM1187 as described in ref. 13 with the following changes. The ammonium sulphate pellet obtained by fractionating the polymin P eluate was extracted with buffer to which 0.20 g ml^{-1}, ammonium sulphate was added; after centrifugation, protein was precipitated from the supernatant by addition of 0.15 g ammonium sulphate per ml. After dialysis, this protein was chromatographed on DNA-agarose, or on phosphocellulose, or on phosphocellulose in 20 mM potassium phosphate, *p*H 6.5, 0.1 mM EDTA, 1 mM dithiothreitol, and 10% (v/v) glycerol, eluting with a gradient of KCl to 0.25 M in the same buffer[9]. Usually we used two cycles of phosphocellulose chromatography, or DNA-agarose followed by phosphocellulose. The peak of protein was concentrated with ammonium sulphate and centrifuged on a sucrose gradient as described in ref. 13, except that the gradient was collected from the top to avoid contamination with polynucleotide phosphorylase, which sediments faster than recA protein. The protein appeared homogeneous by gel electrophoresis in SDS. The main criterion for purity was complete dependence on added polynucleotide for repressor cleavage, a condition that correlated with very low or undetectable activity of polynucleotide phosphorylase. Phosphate was added to reaction mixtures to inhibit any residual polynucleotide phosphorylase. There was no detectable endonuclease active on denatured λ DNA in either recA protein or λ repressor. λ repressor was prepared from *E. coli* 294 (pKB277) (ref. 17) by the following modification of the method of Sauer and Anderegg[18]. Cells were grown in medium containing 16 g tryptone, 10 g yeast extract, and 5 g NaCl per litre to an A_{550} of 1. After fractionation of the crude extract with polymin P and ammonium sulphate, protein was loaded onto a single stranded DNA-agarose column prepared as described by Schaller *et al.*[19], in a buffer containing 10 mM Tris HCl *p*H 7.9, 1 mM EDTA, 2 mM $CaCl_2$, 3 mM dithiothreitol, 5% glycerol (v/v), and 0.1M KCl. Protein was eluted with the same buffer containing 0.4 M KCl, chromatographed on hydroxylapatite[13], concentrated with ammonium sulphate, and centrifuged on a 10–30% (v/v) glycerol gradient in the above buffer containing 10 mM dithiothreitol and 0.2 M KCl for 40 h at 35,000 r.p.m. in the Beckman SW41 rotor. Sometimes the centrifugation was repeated to remove traces of polynucleotide phosphorylase. λ DNA, prepared by phenol extraction, was denatured by heating for 5 min at 100 °C, followed by rapid chilling. ATP-γS was obtained from Boeheringer.

A parenthetical, but important point is that the further purification of recA protein does not separate from it any factors required for repressor cleavage. Thus we presume that recA protein is the protease, although we lack a rigorous proof that recA protein does not act in concert with a protease present in trace amounts that we cannot detect separately. We can conclude that recA protein is directly required for the reaction, because variants of recA protein prepared from mutants display different activities for repressor cleavage *in vitro* (refs 12, 13 and unpublished experiments).

The polynucleotide requirement for repressor cleavage

Since both recA protein and λ repressor are DNA-binding proteins, either or both might interact with polynucleotide in repressor cleavage. The following results suggest that the primary interaction of polynucleotide is with recA protein.

Polynucleotide-dependent ATPase activity of recA protein: Purified recA protein has a polynucleotide-dependent ATPase activity; its dependence on single-stranded DNA is shown in Fig. 4. The characteristics of this ATPase suggest that the same interaction of polynucleotide and ATP with recA protein activates both ATPase and the repressor cleavage reaction. All single-stranded polynucleotides that stimulate repressor cleavage (Table 1) also stimulate the ATPase, except the two oligonucleotides. (These oligonucleotides stimulate repressor cleavage with ATP-γS, but not detectably with ATP; data not shown.) Native DNA is severalfold less effective than denatured DNA in stimulating the ATPase in these conditions (data not shown).

The ATP analogue ATP-γS, which substitutes effectively for ATP in repressor cleavage, is not hydrolysed by recA protein; in

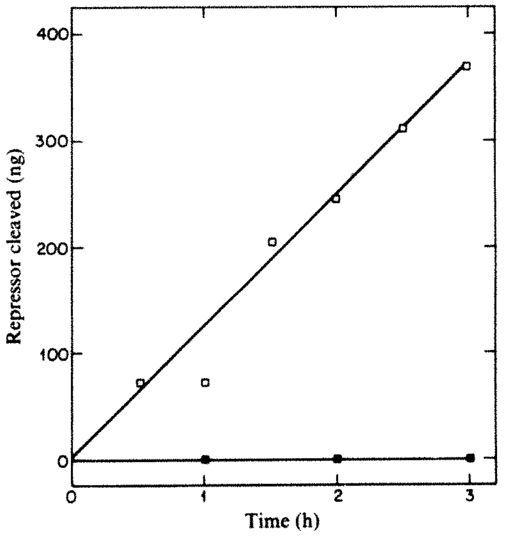

Fig. 2 Time course of λ repressor cleavage. Reaction mixtures of 20 μl were prepared as described in Fig. 1, but containing 0.14 M NaCl, 0.045 M KCl, 2 mM $MgCl_2$, 1 mM ATP-γS, 0.5 μg recA protein, 4.1 μg λ repressor, and 1.4 μg ml^{-1} denatured λ DNA (unless omitted). After incubation reaction mixtures were analysed by SDS gel electrophoresis and staining with Coomassie brilliant blue. The amount of repressor cleaved was measured by densitometric tracing of fragments R_1 and R_2, using as a standard repressor fragments from a reaction mixture in which a known amount of repressor was completely cleaved (as in Fig. 3*c*). ■, DNA omitted; □, complete reaction.

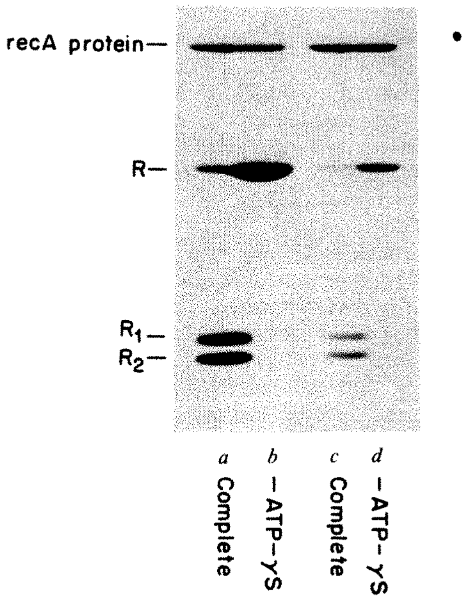

Fig. 3 Efficiency of λ repressor cleavage. Reaction mixtures of 30 μl were prepared as described in Fig. 1, except that they contained 2 mM $MgCl_2$, 1 mM ATP-γS (unless omitted), 0.9 μg recA protein, 1.3 μg ml^{-1} denatured λ DNA and λ repressor as given. After incubation for 5 h at 37 °C, reaction mixtures were analysed as in Fig. 1. Track *a*, complete reaction with 8.4 μg λ repressor; track *b*, as track (*a*), but ATP-γS omitted; track *c*, complete reaction with 1.4 μg λ repressor; track *d*, as track *c*, but ATP-γS omitted.

fact, it is a potent inhibitor of both the ATPase activity (Fig. 4) and the strand invasion reaction[10,11,13]. A reasonable interpretation of these facts is that binding alone of either nucleotide to a site on recA protein in the presence of polynucleotide activates repressor cleavage, whereas hydrolysis of ATP is required for strand invasion. It is consistent with this proposal that for both repressor cleavage and ATPase, ATP is required at a relatively high concentration and ATP-γS is effective at a relatively low concentration. ATP is required at ~1 mM to stimulate repressor cleavage, near its K_m for the ATPase reaction (~0.5 mM) (data not shown). ATP-γS inhibits the ATPase reaction in the micromolar concentration range (Fig. 4), at which it is also effective in supporting repressor cleavage.

Polynucleotide concentration dependence of repressor cleavage: The rate of recA protein-directed cleavage of repressor depends on the concentration of polynucleotide in the reaction mixture. When ATP-γS is used, there is a striking polynucleotide concentration optimum. Figure 5 shows a polyacrylamide gel analysis of reaction mixtures incubated with increasing concentrations of single-stranded DNA. In control experiments, we found the rate of repressor cleavage to be constant during this incubation, so that we can infer the rate of cleavage from the final yield of repressor fragments R_1 and R_2 (see Fig. 2). It is apparent that a maximum rate is reached as the concentration of DNA is increased and that higher concentrations sharply inhibit the reaction. Single-stranded DNA of phage G4, poly(rA), and poly(dT) yield similar concentration optima and maximum cleavage rates. The optimum for duplex DNA is much broader and occurs at a higher DNA concentration. When ATP is used instead of ATP-γS, the concentration optimum for single-stranded polynucleotide is the same, but the inhibition by higher concentrations is less pronounced.

The nature of the DNA concentration dependence of repressor cleavage suggests that the polynucleotide interacts with recA protein: both the maximum rate of cleavage and the DNA concentration at which this maximum occurs vary with the concentration of recA protein. To show this, we have constructed DNA dose curves similar to that in Fig. 5, using three different concentrations of recA protein. From this experiment (Fig. 6) we draw three conclusions:

(1) The DNA concentration optimum is approximately proportional to the recA protein concentration. Thus with 15, 30 and 60 μg ml^{-1} recA protein, the respective DNA concentration optima are about 0.8, 1.5 and 3 μg ml^{-1}. Taking 40,000 and 330 as the molecular weights of a recA monomer[6] and a nucleotide of DNA, we calculate that the maximum rate of cleavage occurs with six nucleotides per recA monomer. (The major uncertainty in this calculation is the concentration of recA protein, which we estimated by assuming an extinction coefficient of 1.0 mg^{-1} ml^{-1} at 280 nm.)

(2) At a DNA concentration less than the optimum, the rate of cleavage is approximately proportional to DNA concentration and independent of recA protein concentration.

(3) The rate of cleavage at the DNA optimum increases with recA protein concentration; for the two higher concentrations of recA protein, this rate is approximately proportional to recA protein concentration.

In similar experiments we have found that the DNA concentration optimum does not change as the repressor concentration is varied.

The active complex of recA protein and DNA

Because the greatest rate of repressor cleavage occurs at a constant ratio of recA protein to DNA for various concentrations of recA protein, we infer that these components form a stoichiometric complex in the presence of ATP or ATP-γS that is active in cleavage. Others have suggested that recA protein binds DNA tightly in the presence of these nucleotides[10,11]. A reasonable presumption is that binding to polynucleotide causes a conformational shift in recA protein to a form that is active to bind and cleave repressors. In this model, the function of ATP or ATP-γS in repressor cleavage might be to promote the formation of the active complex of recA protein and polynucleotide. The repressor also could contact polynucleotide during the cleavage reaction, but we have no information about this possibility.

It seems likely that the recA protein–DNA complex active in repressor cleavage is the same that is active in the strand

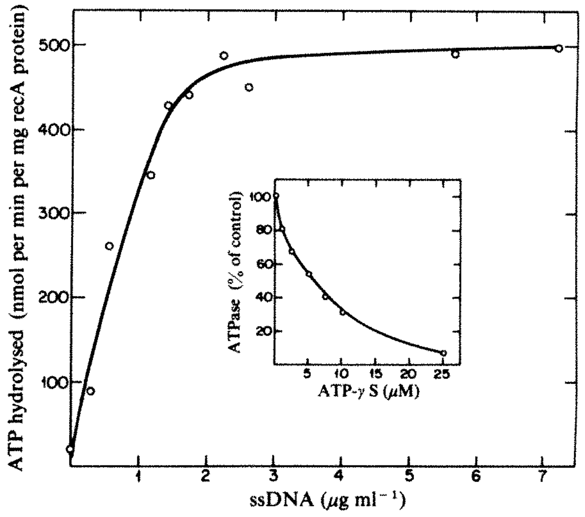

Fig. 4 DNA-dependent ATPase activity of recA protein. (Inset: inhibition of recA ATPase by ATP-γS). Reaction mixtures contained 11 mM Tris-HCl, *p*H 7.5, 0.18 M NaCl, 10 mM $MgCl_2$, 0.5 mM dithiothreitol, 0.35 mM EDTA, 5 mM [γ-^{32}P]ATP, 6.5% (w/v) sucrose, 29 μg ml^{-1} recA protein, and denatured λ DNA as indicated. After an incubation of 15 min at 37 °C, ATP hydrolysis was determined by the method of Avron[22]. Inhibition by ATP-γS was measured in reaction mixtures as above, but containing 27 μg ml^{-1} recA protein, 68 μg ml^{-1} denatured DNA, 1.5 mM [γ-^{32}P]ATP, and ATP-γS as indicated; ATP hydrolysis was determined after an incubation of 30 min at 37 °C.

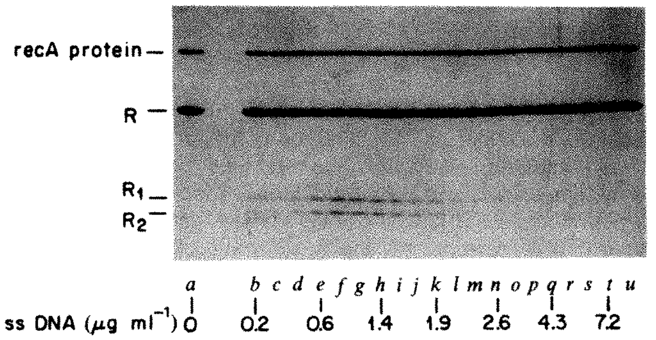
recA protein
R
R1
R2
a b c d e f g h i j k l m n o p q r s t u
ss DNA (μg ml^{-1}) 0 0.2 0.6 1.4 1.9 2.6 4.3 7.2

Fig. 5 Effect of single-stranded DNA concentration on repressor cleavage. Reaction mixtures of 20 µl were prepared as described in Fig. 1, except that they contained 0.14 M NaCl, 0.04 M KCl, 2 mM KCl (ref. 2), 1 mM ATP-γS, 0.5 µg recA protein, 3.7 µg λ repressor, and denatured λ DNA as indicated. After incubation for 2 h at 37 °C reaction mixtures were analysed by SDS-gel electrophoresis and staining with Coomassie brilliant blue. Track *a*, no DNA; tracks *b–u*, 0.2, 0.3, 0.4, 0.6, 0.8, 1.2, 1.4, 1.5, 1.7, 1.9, 2.2, 2.5, 2.6, 3.0, 3.5, 4.3, 5.0, 5.7, 7.2 and 9.5 µg ml^{-1} DNA.

invasion reaction. Shibata *et al.*[8] have determined an optimum ratio of one recA monomer to five nucleotides of single-stranded DNA to catalyse invasion of this strand into duplex DNA to form a D-loop; this is identical within experimental error to our calculated optimum of six nucleotides per recA monomer for the repressor cleavage reaction. Furthermore, D-loop formation also is inhibited if DNA is present in excess over the apparent stoichiometric value[8,10]. An obvious difference between the reactions is that ATP-γS promotes repressor cleavage but not D-loop formation. However, Cunningham *et al.*[11] have shown that ATP-γS does stimulate recA protein to melt duplex DNA in the presence of single-stranded DNA. Thus recA protein is activated both for repressor cleavage and for this initial step of strand invasion by interaction with single-stranded DNA and ATP-γS. (Presumably ATP hydrolysis is required for a subsequent step of strand invasion.)

We do not know why excess DNA inhibits the cleavage and strand pairing activities of recA protein. We note that inhibition of repressor cleavage by excess DNA is most striking in the presence of the ATP analogue ATP-γS, which is not hydrolysed, and that the ATPase activity of recA protein is not inhibited by high concentrations on DNA. One possibility is that excess DNA interacts with a second site of recA protein normally utilised in the strand pairing reaction, and that this interaction locks the protein into an inactive form when the non-hydrolysable analogue is present. As excess single-stranded DNA also inhibits strand invasion, it probably affects recA protein rather than repressor.

Regulation of recA protein activity *in vivo*

A lysogen is induced by treatments that damage cellular DNA, leading to cleavage and inactivation of repressor by recA protein. We have described conditions in which recA protein cleaves λ repressor *in vitro*. How is this activity of recA protein regulated to cause induction? The presence of wildtype recA protein in the cell is not sufficient to cause repressor inactivation *in vivo*. Instead, it is likely that a signal invoked by damage to cellular DNA activates recA protein to cleave repressors[5-7,23]. This activation also is thought to cause amplification of recA protein: by hypothesis, the inducing signal stimulates a small amount of recA protein present before induction to cleave a repressor of the *recA* gene, encoded by the *lexA* gene, leading to amplification of the recA protein itself. Both the activation and the increased amount of recA protein are probably required for full expression of *recA*-dependent processes, including phage repressor inactivation.

How can we relate the requirement for a signal to activate recA protein *in vivo* to the fact that recA protein is active in our conditions *in vitro*? One possibility is that we are providing the signal, or one possible signal, by adding single-stranded DNA. Many inducing treatments (such as UV irradiation) lead to single-stranded regions ('gaps') in chromosomal DNA because replication is interrupted at lesions (such as pyrimidine dimers)[24]; such gaps could bind and activate recA protein and thus be a sufficient signal for induction. It is reasonable that recA protein should be directed to gaps, because its major DNA repair function is probably to remove such discontinuities by provoking recombination of the damaged helix with an intact sister chromatid. Alternatively, oligonucleotides resulting from degradation of damaged DNA could provide a signal to activate recA protein[23].

We doubt, however, that a polynucleotide derived from damaged DNA is the only possible signal. A mutation in the *recA* gene, named *tif*[25], alters the protein to make it active without damage to cellular DNA; it is sufficiently active at high temperature (40° C) to allow full induction of *recA*-dependent functions, and it has substantial activity at low temperature (30° C) in the absence of an inducing treatment[15]. Yet purified *tif*$^-$-recA protein, which we used in these experiments, is completely dependent on polynucleotide for repressor cleavage activity *in vitro*. What polynucleotide does *tif*$^-$-recA protein utilise *in vivo*? One possibility is duplex chromosomal DNA, since native DNA has significant activity to promote repressor cleavage *in vitro* (Table 1). Alternatively *tif*$^-$-recA protein could interact with a unique DNA structure at a growing fork or at the origin of replication[26,27]. In either case, the *tif*$^-$-recA protein might be able to utilise chromosomal DNA under conditions in which the wild type protein cannot. The fact that *tif*$^-$-recA protein appears to utilise some undamaged polynucleotide in the cell suggests a second possible type of inducing signal for recA protein: a regulatory molecule could be elaborated in induced cells that binds recA protein and allows it to interact with chromosomal DNA, so that recA protein is activated both to cleave repressors and to engage in recombination. Further study of the behaviour of recA protein *in vitro* may reveal what signal or signals activate it *in vivo*.

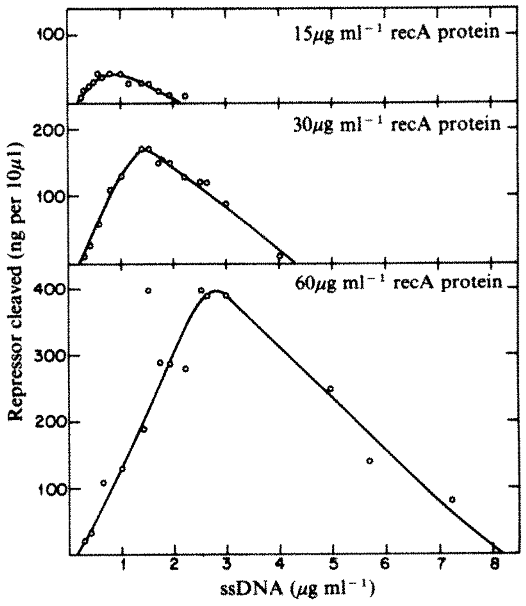

Fig. 6 DNA dependence of repressor cleavage at various concentrations of recA protein. Reaction mixtures prepared as in Fig. 1 contained 0.14 M NaCl, 0.045 M KCl, 2 mM $MgCl_2$, 1 mM ATP-γS, 120 µg ml^{-1} λ repressor, and recA protein and denatured λ DNA as indicated. After incubation for 2 h at 37 °C, reaction mixtures were subjected to SDS gel electrophoresis and cleavage was measured as described in Fig. 2.

Function of the protease activity of recA protein

The protease activity of recA protein clearly is responsible for the induction of temperate bacteriophages, and probably for induction of the *recA* gene itself. Thus it is required at least indirectly for recombinational repair of DNA, which occurs efficiently only if recA protein is amplified. Other *recA*-dependent processes such as mutagenesis might involve proteolytic modification of cellular proteins, as well as the strand pairing activity of recA protein.

Does the protease activity also have a direct role in genetic recombination? The fundamental reaction of recombination catalysed by purified recA protein on naked DNA *in vitro* clearly does not utilise this activity[8-11]. Despite this, it is possible that the protease activity affects the rate of *recA* function *in vivo*. Cellular DNA is complexed with a variety of proteins, so that another role of the protease activity could be to remove such proteins to facilitate the exploration of duplex DNA that must precede strand pairing. An analysis of mutations that affect different activities of recA protein might indicate if this is true.

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Morphogenetic genes *C* and *Nu3* overlap in bacteriophage λ

Jocelyn E. Shaw & Helios Murialdo

Department of Medical Genetics, University of Toronto, Toronto, Canada M5S 1A8

In bacteriophage λ, *genes* C *and* Nu3, *two of the four cistrons which are essential for normal prohead formation, have overlapping nucleotide sequences. These genes are translated in the same reading frame so that the Nu3 protein is identical to the COOH-terminal one-third of the C protein. This structural relationship may provide for the functional interaction of the C and Nu3 proteins through their regions of structural homology during prohead assembly. The in-phase overlapping organisation of genes may constitute a general strategy to facilitate the mutual interaction of a pair of proteins through their common structural domains.*

MORPHOGENESIS of the head of bacteriophage λ is controlled by ten genes clustered in the left arm of the phage chromosome (Fig. 1*a*)[1-7]. Of these genes only *B*, *C*, *Nu3* and *E*, encoding proteins of molecular weights (MWs) 60,000, 58,000, 19,000 and 38,000, respectively, are essential for the production of the head precursor known as the prohead[8-15]. pNu3 acts as the scaffolding protein in prohead construction, directing the assembly of the major capsid protein, pE (refs 14–17). Nu3 protein is subsequently degraded and lost from the prohead structure[14,15,17]. pC is thought to be involved in the early stages of prohead assembly. It is ultimately fused to a minor fraction of the pE molecules in the prohead and cleaved to generate X1 and X2, two polypeptides differing only in the size of their pC-derived moiety. These two proteins, as well as pB and its cleavage product pB*, are the minor components of the mature prohead[14-19].

There is only one available amber mutation in gene *Nu3*—ama8 (ref. 4). Previous examination of the proteins synthesised after infection of non-permissive *Escherichia coli* by $\lambda Nu3_{ama8}$ suggested an unusual relationship between pNu3 and pC (ref. 17). In these studies the mutation in gene *Nu3* resulted not only in the disappearance of pNu3 and the appearance of a Nu3 amber polypeptide, but also in the appearance of a lower MW form of pC. As C protein is known to be susceptible to cleavage[17,18], the production of this altered form of pC was initially attributed to a rapid aberrant cleavage of pC in the absence of functional pNu3 (ref. 17). However, it was noted that the difference in MW between pNu3 and its amber peptide was very similar to the difference in MW between pC and the lower MW form of pC. An alternative explanation for these observations—that the gene products of *C* and *Nu3* have a common COOH terminus—was therefore considered. By this model the

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two proteins are translated in the same reading frame and terminated at the same site. Thus, the amber mutation in gene *Nu3* is also an amber mutation in gene *C* causing premature termination in the translation of both proteins so that the pC and pNu3 amber polypeptides synthesised by $\lambda Nu3_{ama8}$-infected cells differ in size from the respective wild-type polypeptides by the same amount. This model predicts that mutations within the promoter-proximal region of the *C* gene will affect only the C polypeptide, whereas mutations within the overlapping promoter-distal region will affect both pC and pNu3. Further, the Nu3 protein will be identical to the COOH-terminal region of the C protein, and tryptic peptides of pNu3 (except perhaps the NH_2-terminal peptide) should be a subset of those derived from pC. In this article we describe experiments the result of which support the predictions of such a model.

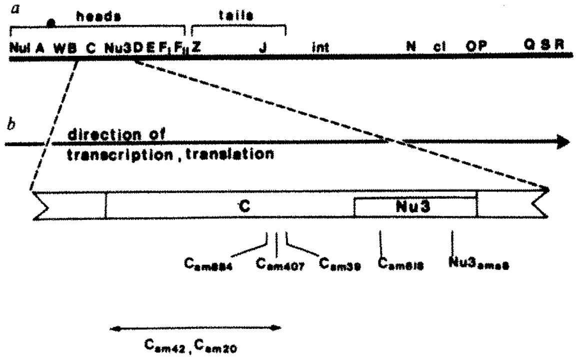

a
heads
tails
Nul A WB C Nu3D E F$_I$ F$_{II}$ Z J int N cI OP Q S R
b
direction of transcription, translation
C
Nu3
C_{am884}
C_{am407}
C_{am39}
C_{am818}
$Nu3_{ama8}$
C_{am42}, C_{am20}

Fig. 1 Genetic map of λ and expanded map of the *C–Nu3* region. *a*, The vegetative map of λ (not to scale) showing the relative positions of the morphogenetic genes involved in head formation, *Nu1–F*$_{II}$, and tail assembly, *Z–J*. *b*, An expanded map of the *C–Nu3* region drawn to scale. Positions of the *C* and *Nu3* genes and of amber sites are determined from the apparent MWs of the corresponding polypeptides and amber fragments as shown in Fig. 2*a*. The relative positions of the mutations are the same as determined genetically. The λC_{am20} and λC_{am42} mutants, whose amber polypeptides were not observed, have been mapped genetically[47], and their amber sites lie to the left of C_{am39}. The direction of transcription and translation is as indicated.

A missense mutation in gene *Nu3* does not alter the MW of the product of gene *C*

As a preliminary step in the investigation of the relationship between pC and pNu3, we compared the MWs of pC and pNu3 synthesised after infection of *E. coli* with either a λ*Nu3* amber mutant or a λ*Nu3* missense mutant. As originally observed by Ray and Murialdo[17], infection of non-permissive cells with $\lambda Nu3_{ama8}$ results in the synthesis of two altered proteins, as seen in SDS-polyacrylamide gels: (1) pNu3 with a MW of 19,000 (ref. 17) is replaced by its amber fragment $pNu3_{a8}$ of approximate MW 15,000, and (2) pC with a MW of 58,000 (ref. 20) is replaced by pC_{a8}(pC* of Ray and Murialdo), with an apparent MW of about 54,000 (Fig. 2*a*). If the production of pC_{a8} by $\lambda Nu3_{ama8}$ were the result of rapid cleavage of a wild-type C protein in the absence of functional pNu3, as previously suggested[17], the C protein made after infection of cells with a different mutant in gene *Nu3* might suffer similar cleavage and be present as a fragment with the same MW as pC_{a8}. This is not the case. $\lambda Nu3_{t78}$, an absolute defective mutant in gene *Nu3* (ref. 5), directs the synthesis of a pC with a MW of 58,000 the same mobility as that produced in cells infected with λcI_{tl} (wild-type λ) (Fig. 2*a*, *d*). Thus, a nonsense mutation, but not a missense mutation in gene *Nu3*, can alter the size of pC. This result favours the model in which *C* and *Nu3* are overlapping genes.

A gene *C* mutant is also a gene *Nu3* mutant

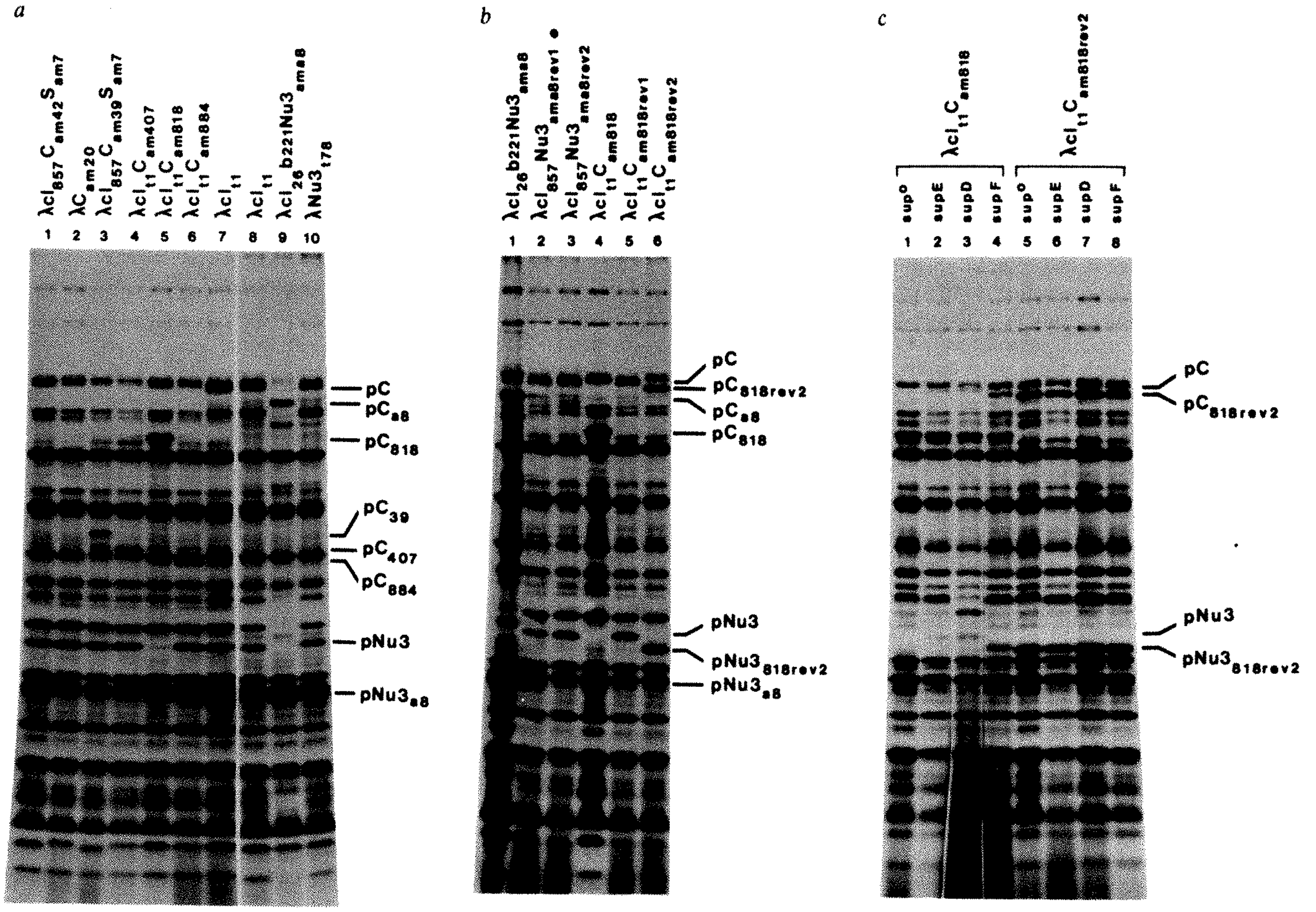
To test the prediction that mutants in the promoter-distal one-third of the *C* cistron will also be mutant in the *Nu3* cistron, we examined the proteins synthesised in non-permissive *E. coli* infected with a series of amber mutants in gene *C* (Fig. 2*a*). For some of these infections, the C amber polypeptide can be resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), as indicated in Fig. 2, and its size can be used to estimate the position of the corresponding amber mutation within the *C* gene. Assuming that genes *C* and *Nu3* terminate at the same site, a map of the *C-Nu3* region of the λ chromosome can be constructed from the MWs of pC and pNu3, and from the MWs of the amber fragments observed (Fig. 1*b*). When this is done, five of the six *C* mutants tested fall into order within the amino-terminal region exclusive to *C*, whereas C_{am818} falls into the region shared by *C* and *Nu3*. As expected, the mutants located in the non-overlapping portion of the *C* gene fail to produce C protein but make normal amounts of Nu3 protein, whereas λC_{am818} makes neither pC nor pNu3. This scheme agrees with genetic data which show that λC_{am818} is the only *C* mutant of this set that fails to complement $\lambda Nu3_{ama8}$ (data not shown).

The electrophoretic mobilities of both pC and pNu3 are altered by insertion of tyrosine at position am818

The above data are consistent with the overlapping genes model but do not exclude the possibility of double mutations in the strains that affect both pC and pNu3, or polarity of the C_{am818} mutation on gene *Nu3*. To confirm that the observed effects are the result of single mutations, spontaneous revertants of $\lambda cI_{857}Nu3_{ama8}$ and $\lambda cI_{tl}C_{am818}$ were selected on strain 594, a non-suppressing host. Revertants were obtained at frequencies typical for single mutations (10^{-7}–2×10^{-7}) and two revertants of each parent were selected for further study. The polypeptides synthesised in non-suppressing hosts infected with these revertants are shown in Fig. 2*b* and *d*. The two revertants of $\lambda cI_{857}Nu3_{ama8}$ and one revertant of $\lambda cI_{tl}C_{am818}$ (rev 1) produce pNu3 and pC of wild-type MW, indicating that the observed alterations of pNu3 and pC, in both $\lambda cI_{857}Nu3_{ama8}$ and $\lambda cI_{tl}C_{am818}$, are due to single mutations in these phages. The other revertant of $\lambda cI_{tl}C_{am818}$, $\lambda cI_{tl}C_{am818rev2}$, directs the synthesis of C and Nu3 proteins each having an apparent MW about 1,000 less than that of wild-type pC and pNu3. This result is another example of a simultaneous and identical alteration in the mobilities of these two proteins due to a single mutational event. Surprisingly, this mobility change of pC and pNu3 in $\lambda cI_{tl}C_{am818rev2}$-infected cells may be due to the particular amino acid that is now inserted at the former amber site. The following experiment was therefore carried out to see if such a change in mobility could arise from the substitution of a single amino acid. Cells carrying different amber suppressors were infected with $\lambda cI_{tl}C_{am818}$ and the mobilities of pC and pNu3 were examined. Infection of *supD* (Ser-inserting suppressor) and *supE* (Gln-inserting suppressor) cells results in the synthesis of small amounts of pC and pNu3 of wild-type size (and also the amber fragment of *C*, Fig. 2*c*). *supF*-infected cells (Tyr-inserting), on the other hand, synthesise C and Nu3 polypeptides having the same mobilities as those found in $\lambda cI_{tl}C_{am818rev2}$-infected cells, that is, polypeptides with an apparent reduction in MW of 1,000. We conclude that it is the insertion of tyrosine at the C_{am818} site that is responsible for the altered mobilities of these polypeptides. The MWs of the C and Nu3 proteins remain unchanged in infections of all three suppressor strains with $\lambda cI_{tl}C_{am818rev2}$. It seems likely that in $\lambda C_{am818rev2}$ the amber codon has been changed to code for Tyr.

The *Nu3*$_{t78}$ mutation alters the isoelectric points of both pC and pNu3

To probe further the model that the C and Nu3 proteins share a common coding region, we investigated the properties of these two proteins synthesised by $\lambda Nu3_{t78}$-infected cells. $\lambda Nu3_{t78}$, as


a
1 $\lambda cI_{857}C_{am42}S_{am7}$
2 λC_{am20}
3 $\lambda cI_{857}C_{am39}S_{am7}$
4 $\lambda cI_{t1}C_{am407}$
5 $\lambda cI_{t1}C_{am818}$
6 $\lambda cI_{t1}C_{am884}$
7 λcI_{t1}
8 λcI_{t1}
9 $\lambda cI_{26}b221Nu3_{ama8}$
10 $\lambda Nu3_{t78}$
pC
pC_{a8}
pC_{818}
pC_{39}
pC_{407}
pC_{884}
pNu3
$pNu3_{a8}$
b
1 $\lambda cI_{26}b221Nu3_{ama8}$
2 $\lambda cI_{857}Nu3_{ama8rev1}$ ●
3 $\lambda cI_{857}Nu3_{ama8rev2}$
4 $\lambda cI_{t1}C_{am818}$
5 $\lambda cI_{t1}C_{am818rev1}$
6 $\lambda cI_{t1}C_{am818rev2}$
pC
$pC_{818rev2}$
pC_{a8}
pC_{818}
pNu3
$pNu3_{818rev2}$
$pNu3_{a8}$
c
$\lambda cI_{t1}C_{am818}$
$\lambda cI_{t1}C_{am818rev2}$
1 sup^{0}
2 supE
3 supD
4 supF
5 sup^{0}
6 supE
7 supD
8 supF
pC
$pC_{818rev2}$
pNu3
$pNu3_{818rev2}$

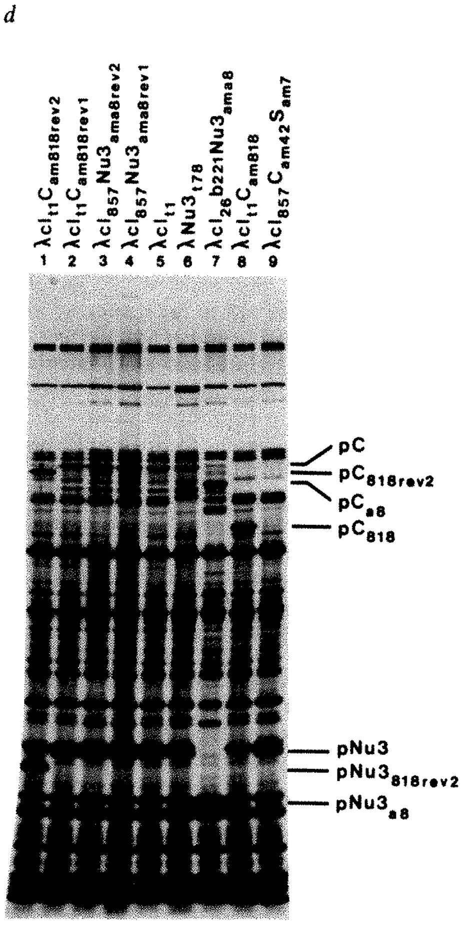

d
1 $\lambda cI_{t1}C_{am818rev2}$
2 $\lambda cI_{t1}C_{am818rev1}$
3 $\lambda cI_{857}Nu3_{ama8rev2}$
4 $\lambda cI_{857}Nu3_{ama8rev1}$
5 λcI_{t1}
6 $\lambda Nu3_{t78}$
7 $\lambda cI_{26}b221Nu3_{ama8}$
8 $\lambda cI_{t1}C_{am818}$
9 $\lambda cI_{857}C_{am42}S_{am7}$
pC
$pC_{818rev2}$
pC_{a8}
pC_{818}
pNu3
$pNu3_{818rev2}$
$pNu3_{a8}$

Fig. 2 Polypeptides synthesised after infection of host cells with mutants in genes *C* and *Nu3*. *a*, Strain 159 (ref. 48) (tracks 1–7) or 159 ($\lambda imm^{434}ind^{-}$)[20,49] (tracks 8–10) was UV irradiated with an incident dose of 500 J m^{-2}, infected at a multiplicity of infection (MOI) of 5 with the indicated phage and labelled at 20–30 min after infection[17] with 75 μCi of ^{35}S-hydrolysate[50] per ml of culture. Radiolabelled polypeptides were separated by SDS-PAGE on a 14–20% gradient gel and visualised by autoradiography. *b*, Strain 159 was UV irradiated, infected with the indicated amber mutants and revertants at an MOI of 5. Proteins were labelled and analysed as in *a*. *c*, Strain 159 (*sup*^{0}), WC5041 (*supE*), WC5043 (ref. 4) (*supD*) or 159*supF* (ref. 51) (*supF*) was UV irradiated, infected at an MOI of 5 with $\lambda cI_{t1}C_{am818}$ or $\lambda cI_{t1}C_{am818rev2}$ as indicated. Proteins were labelled and analysed as in *a*. *d*, Strain 159 was UV irradiated, infected with the indicated phage at an MOI of 5. Proteins were labelled as in *a* and separated by SDS-PAGE on a 12.5% gel. For SDS-PAGE, extracts were boiled in SDS-gel electrophoresis sample buffer for 1 min and the radiolabelled polypeptides electrophoresed using the discontinuous buffer system of Laemmli and Maizel[52] with the slab gel apparatus described by Studier[53] modified to accommodate 20-cm separating gels. The acrylamide concentration in the separating gels was either 12.5% or a linear gradient of 14–20%. Although separating gels containing 12.5% acrylamide allow better resolution of pC, 14–20% gradient gels are, in general, required for resolution of pNu3.

mentioned above, is an absolute defective mutant in gene *Nu3* that produces C and Nu3 proteins of wild-type MW. Extracts of cells infected with this mutant were analysed by two-dimensional isoelectric focusing and SDS-PAGE[21]. Comparison of the two-dimensional pattern of polypeptides from λcI_{tl}- and $\lambda Nu3_{t78}$-infected hosts reveals that both $pNu3_{t78}$ and pC_{t78} are more basic than the respective wild-type proteins (Fig. 3). This is most clearly seen when samples from extracts of λcI_{tl}- and $\lambda Nu3_{t78}$-infected cells are mixed and run together in the same gel (Fig. 3*c*). All other proteins synthesised in the mutant infection have the same mobilities as in a wild-type infection, with the exception of three minor proteins of slightly lower MW than pC which are polypeptides derived from C protein (pC-associated minor bands of Ray and Murialdo)[17].

The tryptic peptides of pNu3 are a subset of the tryptic peptides of pC

^{35}S-labelled proteins C and Nu3 and polypeptides pC_{a8} and $pNu3_{a8}$ were isolated by SDS-PAGE, eluted from the gel, digested with trypsin and the resulting peptides separated by electrophoresis and chromatography on cellulose thin layer plates[22]. The radiolabelled tryptic peptides of pNu3 are all contained within the peptide map of pC (Fig. 4), indicating that these two proteins have common amino acid sequences. In addition, the tryptic peptide missing (peptide 1) from the fingerprint of the amber polypeptide $pNu3_{a8}$ is also absent in the peptide map of pC_{a8}. This indicates that the $Nu3_{am8}$ mutation results in the production of amber polypeptides of C and Nu3 both lacking the same COOH-terminal amino acid sequence, and demonstrates that the Nu3 protein and the COOH-terminal portion of the C protein are encoded by the same DNA.

Conclusions and implications

The results presented here demonstrate that the Nu3 protein and the COOH-terminal one-third of the C protein are encoded on the same DNA. The Nu3 protein is not derived from pC by proteolytic processing because all the *C* amber mutants located in the NH_2-terminal (non-overlapping) portion of the gene direct the synthesis of normal amounts of Nu3 protein. It is possible that C protein is not a primary gene product but arises from the fusion of Nu3 protein with a polypeptide encoded in the region immediately preceding gene *Nu3*. However, this seems unlikely as co-infection of cells with *C* amber and *Nu3* amber mutants does not result in the production of pC with wild-type MW. Furthermore, pulse–chase experiments fail to provide any evidence of a short-lived precursor to pC (data not shown). We therefore believe that the *Nu3* gene lies within the *C* gene. Translation of the two proteins is in the same reading frame and termination of translation occurs at or near the same site.

It is not known whether translation of the Nu3 protein is initiated internally to C on a common mRNA or whether the two proteins are translated separately from two different mRNAs. In two other instances of overlapping genes in which this has been investigated—the polyoma proteins VP2 and VP3 (refs 23, 24), and A and A* proteins of ΦX174 (ref. 25)—all the proteins seem to be translated from distinct species of mRNA. In the case of the C and Nu3 proteins of λ, there is no evidence to exclude the possibility of translation from either a common mRNA or separate RNAs. Although there is probably not a separate promoter for the transcription of the *Nu3* gene[26], it is possible that the primary polycistronic late transcript initiated from P'_R (ref. 27) is modified so that pC and pNu3 are translated from different species of mRNA. On the other hand, the functional halflife of the *C* mRNA is about half that of the *Nu3* mRNA[28], which is compatible with their being translated from the same transcript. Also, about two- to threefold more copies of pNu3 than pC are synthesised in an infected cell (data not shown), suggesting that there is a relatively strong ribosome binding site for *Nu3* within the *C* gene. This might allow internal initiation for the translation of pNu3 on a common transcript. In any event, we have observed that translation of the C protein probably interferes with translation of pNu3. In cells infected with λC_{am737}, the most promoter-proximal amber mutation in the *C* gene, the amount of Nu3 protein synthesised is about 1.5–2 times as much as is produced in a wild-type infection (data not shown). Although probably not important in the case of the C and Nu3 proteins of λ, interference in the synthesis of the promoter-distal protein by the translation of the promoter-proximal polypeptide could be a means of regulating the level of the downstream protein in an overlapping gene pair[29–32].

There are several examples of overlapping genes in the small double-stranded bacteriophages ΦX174 (refs 32–35) and G4 (refs 36, 37) in the RNA phage *Qβ* (refs 38–40) and in the

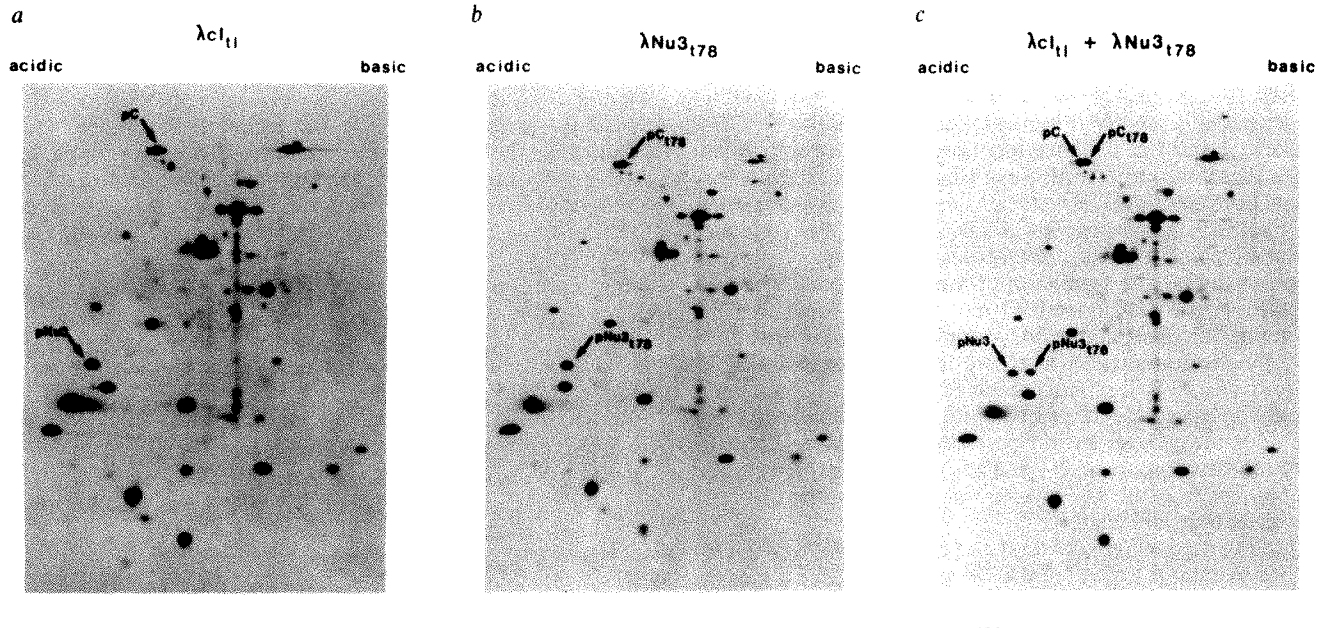


Fig. 3 Two-dimensional separation of proteins synthesised in $\lambda Nu3_{t78}$- infected cells. Strain 159 ($\lambda imm^{434} ind^-$) was UV irradiated, infected with either λcI_{tl} or $\lambda Nu3_{t78}$ at an MOI of 5, and labelled with ^{35}S-hydrolysate at 20–30 min after infection. The proteins were separated in the first dimension by isoelectric focussing in gels containing 2% ampholytes (*w*/*v*) (20% *p*H 3.5–10, 80% *p*H 5–8) and in the second dimension by SDS-PAGE on a 14–20% gradient gel[21]. Radiolabelled polypeptides were visualised by fluorography[54]. *a*, λcI_{tl}; *b*, $\lambda Nu3_{t78}$; *c*, 1:1 (v/v) mixture of samples in *a* and *b*.

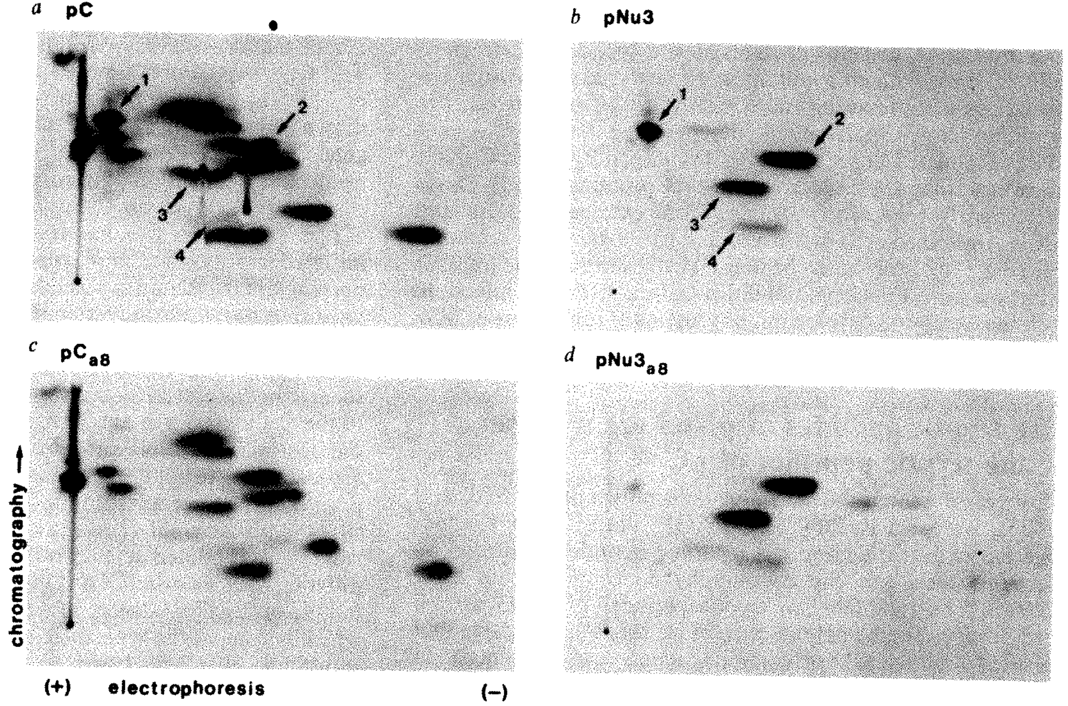


Fig. 4 Tryptic fingerprints of pC, pNu3, pC$_{a8}$ and pNu3$_{a8}$. Strain 159 was UV irradiated and infected with either $\lambda cI_{26}b221E_{am815}$ for preparation of pC and pNu3 or $\lambda cI_{857}Nu3_{ama8}FI_{am785}$ for preparation of pC$_{a8}$ and pNu3$_{a8}$. Use of these particular phage strains allows isolation of pNu3 and pNu3$_{a8}$ relatively free of contaminating labelled phage proteins. The infected cells were labelled at 20–30 min after infection with ^{35}S-hydrolysate and proteins were separated by SDS-PAGE on a 12.5% gel. Radiolabelled polypeptides were visualised by autoradiography and pC, pNu3, pC$_{a8}$ and pNu3$_{a8}$ were cut out of the gel, eluted, oxidised with performic acid, and digested with trypsin[22]. Analysis of the isolated but undigested proteins by SDS-PAGE on a 14–20% gradient gel indicated that pC, pNu3 and pC$_{a8}$ were free of contaminating labelled proteins. pNu3$_{a8}$ contained minor amounts of contaminating material. The tryptic peptides were separated by electrophoresis and chromatography on cellulose thin layer plates[22]. Peptides from pC(*a*) and pNu3 (*b*) were run simultaneously, in parallel, on one thin layer plate, as were peptides from pC$_{a8}$ (*c*) and pNu3$_{a8}$ (*d*). Peptides derived from pC and pNu3 having identical mobilities are indicated by arrows.

papovaviruses polyoma[41] and SV40 (refs 42, 43). It has been argued that the overlapping organisation of genes in these viruses offers the advantage of efficient use of a limited coding capacity. From this viewpoint, the discovery of an overlapping pair of genes in λ may be somewhat surprising, as λ has a relatively large genome with a large non-essential region which itself may contain an overlapping gene pair (C. Epp and M. Pearson, personal communication). This may indicate that an overlapping genetic arrangement is a fairly widespread phenomenon with additional advantages to the organism. The fact that pC and pNu3 are translated in the same reading frame suggests that the role of the overlap, in this instance, is to generate a pair of proteins with a specific structural relationship essential for proper prohead assembly. The overlapping arrangement of *C* and *Nu3* ensures that pNu3 and the COOH-terminal one-third of pC have identical amino acid sequences and are therefore structurally very similar, whereas the NH$_2$-terminal portion of pC is structurally unique. A possible model for λ prohead morphogenesis is one in which the common domains of pNu3 and the COOH-terminal region of pC copolymerise during the formation of a prohead core structure. Molecules of pC thus assembled into the core could further interact through their unique NH$_2$-terminal domains with other morphopoietic proteins such as pE, during the polymerisation of the capsid. (An NH$_2$-terminal portion of pC is known to interact with pE, the major capsid protein, and by fusion with pE and cleavage of the pC–pE hybrid polypeptide produces the two minor prohead components X1 and X2 (ref. 15).) In this way, pC might be viewed as a linker between two different substructures, the prohead core and coat.

Of the overlapping genes translated in phase that have been described to date (*A* and *A** of ΦX174 and G4, *VP2* and *VP3* of the papovaviruses, and A_1 and *coat* of *Qβ*), the VP2 and VP3 proteins of polyoma and SV40, and the A_1 and coat proteins of *Qβ* (ref. 44) are essential for viral morphogenesis. The VP2 and VP3 proteins, organised genetically in an analogous fashion to pC and pNu3 of λ, have common COOH-terminal regions whereas the coat protein and A_1 protein, a readthrough product of the *coat* gene, have identical NH$_2$-terminal sequences. In polyoma, recent experiments suggest that VP2 and VP3 form the pentons of the capsid[45]. This is consistent with the model that these proteins interact with one another because of their common amino acid sequence. Thus, the in-phase overlapping arrangement of genes could be a general strategy to generate a pair of proteins, such as pC and pNu3, with regions of structural homology and non-homology. The structural relationship between the proteins of such a pair may result in their mutual interaction through the shared structural domain[46] while allowing individual specialised reactions through their unique structural domains. In this way, interactions involving each protein of the pair could be physically coupled to facilitate the assembly of a complex biological structure.

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Amino acid sequence of homogeneous antibodies to dextran and DNA rearrangments in heavy chain V-region gene segments

J. Schilling*, B. Clevinger†, J. M. Davie† & L. Hood*

* Division of Biology, California Institute of Technology, Pasadena, California 91125
† Department of Microbiology and Immunology, Washington University School of Medicine, St. Louis, Missouri 63110

The complete variable region sequences from ten antibodies and two myeloma proteins binding α-1,3 dextran have been determined. The diversity patterns of these homogeneous antibody molecules suggest that the variable regions of heavy chains are encoded by separate variable (V) and joining (J) gene segments. The most striking feature of these data is the extensive sequence variability of a region that we denote the D (diversity) segment which is located at the junction between the V and J segments in the centre of the third hypervariable region. The D segment diversity may arise from a novel somatic mutational mechanism or may be encoded by multiple D gene segments. For the first time, the amino acid sequence correlates of several V region idiotypes are determined.

STUDIES of immunoglobins at the protein and DNA levels have led to striking advances in our understanding of the organisation and evolution of antibody genes and mechanisms of antibody diversity[1–4]. There are three unlinked families or clusters of antibody genes—the λ and κ gene families encode light (L) chains and the heavy (H) gene family encodes heavy chains[5]. The κ or λ gene is composed of four distinct gene segments, leader (L), variable (V), joining (J) and constant (C), which are separated by intervening sequences in undifferentiated or germ-line DNA[6–12]. Heavy chain genes probably have a similar organisation of gene segments (P. Early, H. Huang, M. Davis and L.H., unpublished observations). During the differentiation of antibody-producing cells, the V_L and J_L gene segments are juxtaposed and together encode the variable or antigen-binding domain of the antibody molecule. (The term V or J segment will refer to the corresponding protein sequence; the term V or J gene segment will refer to the corresponding DNA coding regions.) Comparative amino acid sequence analyses of V regions showed that for both light and heavy chains, there are three regions of extreme variability termed hypervariable regions[13,14], which fold in three dimensions to constitute the walls of the antigen-binding site[15,16]. The diversity in the hypervariable regions of L chains has been most extensively studied and explained by a variety of genetic mechanisms. (1) There are many germ-line V_κ (and J_κ) gene segments[5,10]. (2) A single V_κ gene segment may be joined to many different J_κ gene segments and conversely, a single J_κ gene segment may be joined to many different V_κ gene segments[17]. As the boundary of the V_L and J_L segments falls within the third hypervariable region, the combinatorial joining of these segments contributes to the diversity of antigen-binding sites. (3) Random single base mutations may occur throughout the V_L (and J_L) gene segments and those variants exhibiting altered and useful antigen-binding properties may be selected for clonal expansion. For example, 12 of 18 V_λ regions examined were identical to one another[18–20]. The six variant V_λ sequences differed from the 12 identical sequences by between one and three amino acid substitutions. All these substitutions occur in the hypervariable regions. These data suggest that a single V_λ gene is often expressed unchanged and less frequently is modified by somatic mutation to produce the variants. Recent DNA sequence analyses of germ-line and myeloma λ gene segments are consistent with this supposition[6,11,12]. The pattern of diversity wherein variant V region sequences exhibit few substitutions mostly concentrated in the hypervariable regions will be denoted λ-type diversity. (4) A site-specific recombinational mechanism has been postulated to explain amino acid sequence diversity at the junction of V_κ and J_κ gene segments[21–23]. This diversity is reflected as single codon substitutions at the N-terminal residue of the J_κ segment and as the insertion or deletion of single codons precisely at the V-J junction[23]. This pattern of diversity will be denoted the V-J junctional diversity.

We have analysed the diversity patterns in mouse antibodies directed against a simple antigenic-determinant, α-1,3 dextran, in order to determine the relative contributions of the diversification mechanisms discussed above. The hybridoma technique of Köhler and Milstein facilitates the analyses of antibody diversity by making large quantities of antigen-induced, homogeneous antibody molecules available[24]. This is accomplished by fusing normal antibody-secreting cells with mouse plasmacytoma cells[25]. The hybrid cells generate immortal cell lines that secrete large amounts of homogeneous antibody molecules of the two parental cell types. The light chains of the dextran antibodies are all of the λ type and appear to be very similar, if not identical, to one another. Hence, most of the diversity in the dextran system appears to reside in the

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Table 1 RNA codons of positions 100 and 101 from V_H regions of myeloma and hybridoma proteins binding dextran*

V–J segments†	Proteins	Codons‡ 100 abc	Codons‡ 101 abc
V_1–J_1	M104E	UAY	GAY
	J558	AGZ CGX	UAY
	Hdex2	AAY	UAY
	Hdex3	AGZ CGX	GAY
	Hdex7	GCX	GAY
	Hdex6	AGY UCX	CAY
V_2–J_1	Hdex8	UAY	GAY
V_3–J_1	Hdex9	AGZ CGX	UAY
V_4–J_1	Hdex10	GUX	AAY
V_1–J_2	Hdex4	AAZ	GAY
	Hdex5	AGY UCX	AAY
V_1–J_3	Hdex1	AAY	UAY

* These codon assignments are derived from the corresponding amino acid sequences

† The V and J segments are defined in Fig. 2.

‡ Y denotes pyrimidine; Z designates purine; and X denotes any one of the four bases.

heavy chains. We report here the complete amino acid sequences from the variable regions of 10 heavy chains derived from hybridoma antibodies to dextran. These data suggest that heavy chains have a D (diversity) segment located in the heart of the third hypervariable region. The D segment may be encoded by a separate germ-line gene segment or generated by a new type of somatic mutational mechanism.

Immunoglobulins binding dextran

Immunoglobulins binding dextran exhibit limited heterogeneity in several respects. First, preliminary amino acid sequence studies on two myeloma proteins binding α-1,3 dextran, M104E and J558, revealed that the V_L regions are identical[18,19] and that the V_H regions are identical through their first hypervariable regions[26]. The N-terminal sequences of V_H regions derived from normally-induced pooled serum antibodies to α-1,3 dextran also are identical to their myeloma counterparts[27]. Second, isoelectric focusing analyses of anti-dextran antibodies of the IgG class demonstrate limited heterogeneity with considerable sharing of bands between sera from different mice[28]. Moreover, the serum antibody light chains are identical to one another and to their myeloma counterparts by isoelectric focusing criteria[27]. Third, the light chains from the 10 hybridomas and 2 myeloma proteins reported here are all of the λ type and have identical isoelectric focusing patterns (J.S., unpublished observation). Thus, light chains from dextran antibodies appear to be very similar, if not identical, to one another. Therefore, most of the diversity in the antibodies to α-1,3 dextran must reside in the heavy chains. Fourth, the anti-α-1,3 dextran antibodies can be divided into several general categories by idiotypic analyses[29]. More than half of the anti-α-1,3 dextran antibodies from mice immunised with the branched dextran B1355 share a variable-region antigenic determinant or idiotype with both the M104E and J558 proteins. This idiotype is absent from non-dextran binding immunoglobulins and λ light chains and is denoted the 'cross-reactive' or IdX idiotype. Antibodies with the IdX idiotype are synthesised by multiple different antibody-producing clones. Additional idiotypes specific for either the M104E or the J558 immunoglobulins are present on a minor fraction of the normally-induced antibodies. These idiotypes are denoted as individual or IdI idiotypes (for example, IdIM104E and IdIJ558). Another general category of dextran antibodies is that of those lacking the IdX determinant (IdX negative). The IdX-positive and IdX-negative antibodies to dextran are structurally very similar in that their light chains are identical by isoelectric focusing analysis and the heavy chains from both the IdX-positive and IdX-negative pools of serum antibodies are identical to the heavy chains of the M104E and J558 immunoglobulins for their N-terminal 27 and 53 residues, respectively[27]. These observations show that much of the V domain diversity in normal antibodies to α-1,3 dextran resides in the heavy chain beyond residue 53. Thus, we turned to the amino acid sequence analysis of homogeneous normal antibodies to α-1,3 dextran.

Diversity patterns in V_H regions from antibodies binding dextran

Figure 1 gives the complete amino acid sequences of V_H regions from 2 myeloma proteins and 10 hybridoma antibodies that bind dextran. The hypervariable regions and the variable region site of carbohydrate attachment are indicated. These V_H regions are compared to the published sequence of the myeloma M104E V_H region[30] with amino acid substitutions indicated by the one-letter code[31].

Two features are of interest in this set of V_H regions. First, each of these 12 V_H regions differs from all of the others by 1 to 13 amino acid substitutions (Fig. 1). We have not yet detected a pair of identical V_H regions in 12 attempts. Accordingly, the V_H sequence diversity in the antibody response to α-1,3 dextran is extensive. Second, patterns of variability divide the V_H regions from dextran antibodies into three distinct sections—V(1–99), V(100–101) and V(102–117). The highly variable section, V(100–101), separates the other two sections which are relatively conserved. Section V(100–101) exhibits nine different sequences in the 12 V_H regions (Fig. 2). Indeed, position 100 has eight alternatives and position 101 has four. In contrast, nine V_H regions have identical sequences for section V(1–99) and the remaining V_H regions differ by between two and seven residues from the others. Likewise, nine V_H regions have identical sequences for the section V(102–117), two have a second sequence (Hdex4 and Hdex5) and one has a third sequence (Hdex1). Thus, the relatively conserved sections V(1–99) and V(102–117) are separated by two highly variable residues at positions 100 and 101.

This same three-section pattern is seen when 20 completely sequenced V_H regions are aligned by homology with their anti-dextran counterparts (Fig. 3). The section homologous to V(1–99) can clearly be defined in each of these 20 V_H regions and each terminates precisely at the position homologous to 99 in the heavy chains binding dextran (Fig. 3). The homology relationships among the V(102–117) sections are also obvious. The V(102–117) sections from the various V_H regions are identical in size, apart from the levan-binding immunoglobulins, and exhibit extensive amino acid sequence homology to one another (56–100% sequence identity). Accordingly, sections homologous to V(1–99) and V(102–117) in the dextran V_H regions are clearly present in all of the other V_H regions examined to date. In contrast, there is no homology in the V(100–101) regions between these two sections because of extensive sequence and size variability (Fig. 3). The sections analogous to V(100–101) can be defined only as those residues remaining after the V(1–99) and V(102–117) sections have been assigned for each V region by homology.

Implications of V_H diversity patterns for gene organisation

The patterns of amino acid diversity in the V_H regions from antibodies binding α-1,3 dextran allow us to define tentatively V_H and J_H segments with properties similar to their light chain counterparts (Figs 1–3). The V(1–99) section appears to correspond to the V_H segment and V(102–117) section appears to

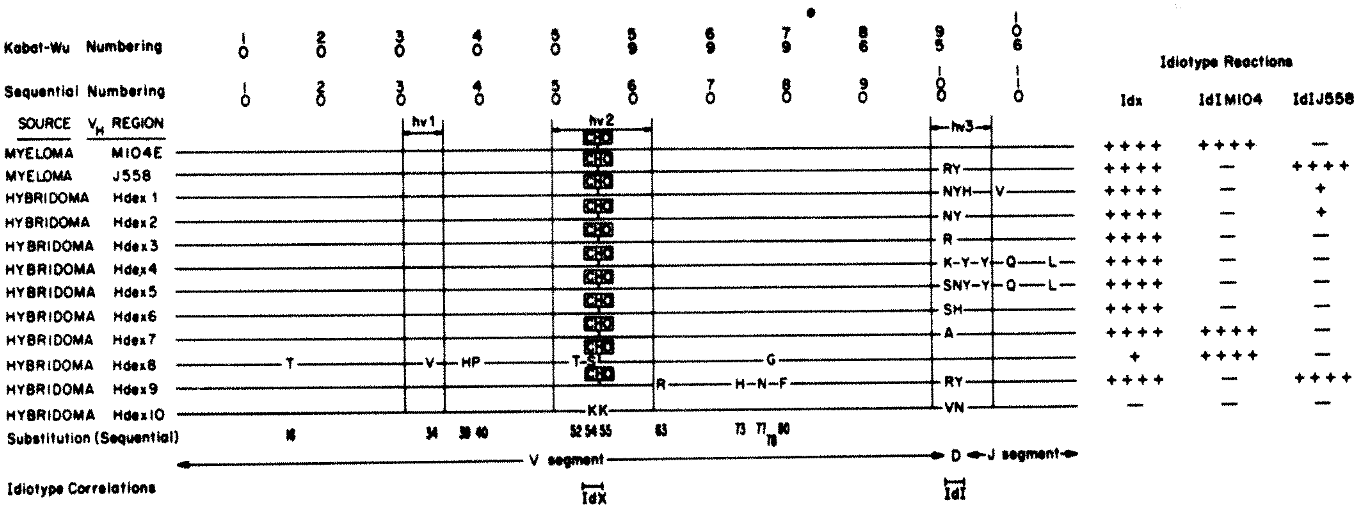


Fig. 1 The amino acid sequences and idiotypes from V regions of myeloma proteins and homogeneous hybridoma antibodies binding dextran. The myeloma protein M104E is used as a prototype sequence and variation from this sequence is indicated. The hypervariable regions are indicated by hv1, hv2 and hv3, respectively. CHO indicates the attachment of a carbohydrate moiety. The one-letter amino acid code of Dayhoff is used[31]. The V and J segments are indicated by arrows. The D segment includes positions 100 and 101. Sequential numbering denotes consecutive numbering of the V_H regions. The Kabat–Wu numbering is from ref. 36. Details of the complete heavy chain sequence of the M104E immunoglobulin are published elsewhere[30]. A similar strategy was used for the sequence analysis of the V_H regions from the hybridomas. The positions of amino acid substitutions in V segments are indicated. Eight hybrids were derived from individual BALB/c mice: Hdex6, 7, 8 and 9 were obtained at 5 or 6 days after a single injection of 100 μg dextran B1355 in complete Freund's adjuvant (CFA), and Hdex2, 3, 4 and 10 were obtained 1–3 days after hyperimmunisation with dextran in CFA followed 1–2 months later with 3 interperitoneal injections of 2×10^9 *Escherichia coli* at 2-day intervals. Hybrids Hdex1 and 5 were derived from a single (BAB-14 × BRVR)F_1 mouse at 7 days after the dextran–*E. coli* protocol. Hybrids were generated as described by Galfre *et al.*[41] using the HGPRT-deficient MPC-11 ($\gamma_{2b}\kappa$) line, 45.6TG1.7, (Hdex1, 3, 4, 5 and 10) or a non-secreting variety of the MOPC-21 ($\gamma_1\kappa$) line, NSI/1-Ag4-1, which synthesises but does not secrete κ chains (Hdex2, 6, 7, 8 and 9). Anti-dextran secreting hybrids were detected by direct binding of ^{125}I-dextran by culture supernates either in a tube-binding assay or after isoelectric focusing in polyacrylamide gels. Hybrids were cloned in soft agar[42] over feeder layers and grown in BALB/c mice as ascites tumours. The antibodies produced by the hybrids appeared representative of those found in the serum at the time of fusion. Idiotypes were determined as described by ref. 29. ++++ Indicates a reaction 10 times as strong as +; – indicates no reaction.

represent the J_H segment. The V(100–101) section will be called the D (diversity) segment and its origin will be discussed.

The extensive variability in these V_H regions at position 100 appears to mark the C-terminal end of the V_H segment, just as extensive variability at position 96 marks the C-terminal end of the V segment in kappa light chains[17]. The correspondence of V(1–99) to a V_H gene segment is confirmed by the DNA sequence analysis of a germ-line V_H gene segment which ends precisely at codon 99 (P. Early, H. Huang, M. Davis and L.H., unpublished observation).

The J_H segment includes at least positions 102–117. The C-terminal boundary of the J_H segment at position 117 has been defined by the DNA sequence analysis of an expressed V_H-J_H gene (H. Huang, P. Early, M. Davis and L.H., unpublished observations). This analysis demonstrates that the C_H region begins at a position homologous to residue 118 in the heavy chains from antibodies binding dextran, in agreement with the J_H and $C_{\gamma1}$ gene segment boundaries defined by DNA sequence analysis of a $C_{\gamma1}$ gene[32]. The N-terminal boundary of the J_H segment is unclear. Figure 2 shows three J_H sequences which differ from each other by two or three two-base substitutions (J_1, J_2 and J_3). Position 102 is the first position in the J_H segment at which linkage relationships among amino acid residues appear. A tryptophan at position 102 is invariably linked to valine, alanine and valine residues at positions 106, 109 and 113, respectively, in the J_1 segments, whereas the tyrosine at position 102 is linked to tyrosine, glutamine and leucine at these positions in the J_2 segments (Fig. 2). These linkage relationships imply that the J_1 and J_2 segments are encoded independently in the germ-line. The D segment comprises positions 100 and 101 and may or may not be a part of the J segment, depending on how V–J junctional diversity arises. These assignments of the V_H, J_H and D segment boundaries also are supported by the homology relationships exhibited among the other completely sequenced V_H regions (Fig. 3).

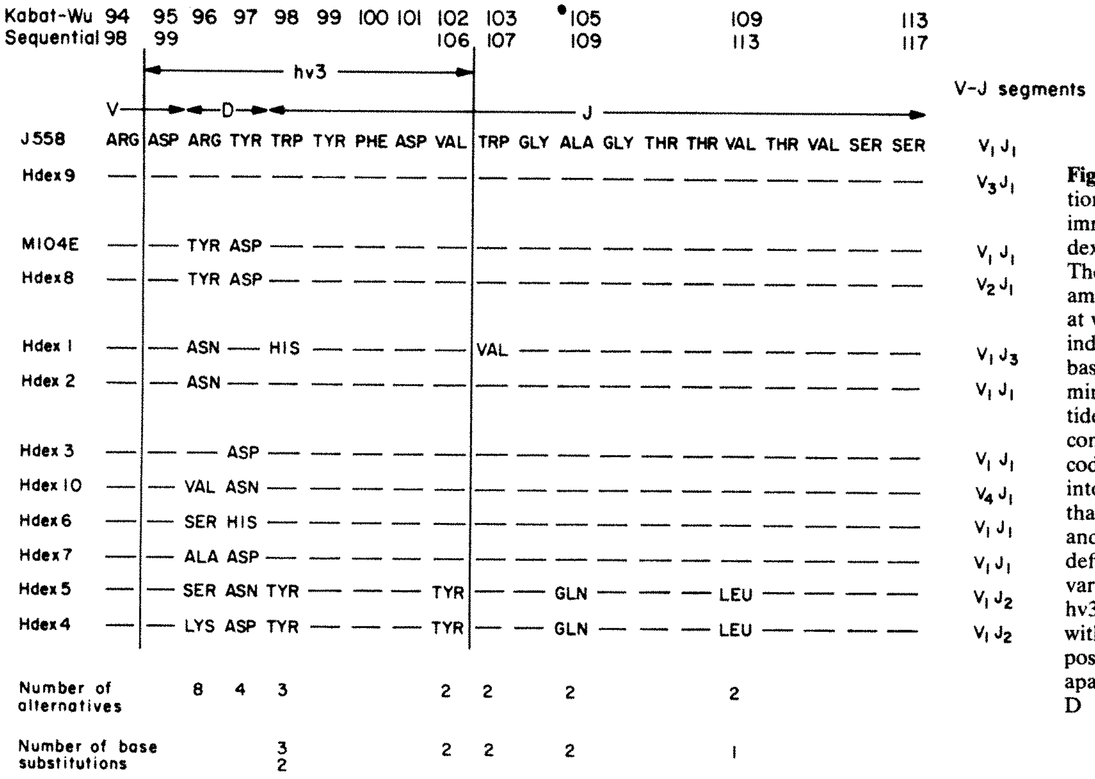
The D and J_H segments comprise almost the entire third hypervariable region (Fig. 2). This is in striking contrast to the J_κ segment which just extends into the third hypervariable region by a single residue[17]. Moreover, the size variation seen in the D segment of heavy chains is far more extensive than that seen at the V–J boundary of kappa chains.

Rudikoff and coworkers have used more limited V_H region data to suggest the V_H segment extends from 1 to 98 and the J_H segment from 105 to 117. They conclude that positions 99 to 104 may be part of the V segment or, alternatively, part of the J segment[33]. These assignments are different from those made here.

Implications of V_H diversity patterns for mechanisms of antibody diversity

The V_H regions from dextran antibodies all differ from one another. Let us analyse possible contributions to this diversity by multiple germ-line gene segments, somatic mutation and the combinational joining of V_H and J_H segments.

Germ-line V_H and J_H gene segments. Four different amino acid sequences corresponding to the V_H gene segment are present in the 12 antibodies binding dextran and these are denoted V_1 to V_4 in Figs 1 and 2. The V_1 segment is expressed in nine different immunoglobulins binding dextran and, accordingly, appears to be encoded by a germ-line gene. This supposition follows from the improbability of generating the same V_H sequence many times by somatic mutation[4]. The V_2, V_3 and V_4 segments differ from V_1 by seven, four and two residue substitutions, respectively. The V_2, V_3 and V_4 segments may be encoded by distinct germ-line V_H gene segments or these sequences may arise from one or more somatic mutational mechanisms. However, the diversity patterns among the V_2, V_3 and V_4 segments do not resemble either of the two somatic mutational patterns described earlier for light chains, λ-type diversity and V_κ-J_κ junctional diversity.



Kabat-Wu	94	95	96	97	98	99	100	101	102	103		•105				109				113	
Sequential	98	99							106	107		109				113				117	V-J segments
J558	ARG	ASP	ARG	TYR	TRP	TYR	PHE	ASP	VAL	TRP	GLY	ALA	GLY	THR	THR	VAL	THR	VAL	SER	SER	$V_1 J_1$
Hdex9	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_3 J_1$
M104E	—	—	TYR	ASP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_1 J_1$
Hdex8	—	—	TYR	ASP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_2 J_1$
Hdex 1	—	—	ASN	—	HIS	—	—	—	—	VAL	—	—	—	—	—	—	—	—	—	—	$V_1 J_3$
Hdex 2	—	—	ASN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_1 J_1$
Hdex 3	—	—	—	ASP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_1 J_1$
Hdex 10	—	—	VAL	ASN	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_4 J_1$
Hdex6	—	—	SER	HIS	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_1 J_1$
Hdex7	—	—	ALA	ASP	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	$V_1 J_1$
Hdex5	—	—	SER	ASN	TYR	—	—	—	TYR	—	—	GLN	—	—	—	LEU	—	—	—	—	$V_1 J_2$
Hdex4	—	—	LYS	ASP	TYR	—	—	—	TYR	—	—	GLN	—	—	—	LEU	—	—	—	—	$V_1 J_2$
Number of alternatives			8	4	3				2	2		2				2					
Number of base substitutions					3 2				2	2		2				1					

Fig. 2 The C-terminal portions of V_H regions from immunoglobulins binding dextran. See Fig. 1 legend. The number of alternative amino acids for each position at which variability exists are indicated. The number of base substitutions denotes the minimum number of nucleotide changes required to convert the most common codon at a particular position into the alternative codons at that position. The V_1 to V_4 and J_1 to J_3 segments are defined. The third hypervariable region is denoted by hv3. Three pairs of proteins with identical sequences at positions 100 and 101 are set apart from the rest. The V_H, D and J_H segments are defined in Fig. 1.

The three different J_H segments differ from one another by two to five residue alternatives (Fig. 2). Moreover, most of these J_H segment substitutions require two or even three base substitutions and repeats of two of these sequences have been seen. These observations strongly suggest that the three J_H sequences are directly encoded by three germ-line J_H gene segments. The J_H diversity noted in Fig. 3 appears to be significantly greater than the corresponding J_L diversity[17] and, accordingly, may be encoded by a larger number of germ-line J_H gene segments.

Combinational joining. The combinational joining of V_H and J_H gene segments to encode many distinct V_H regions provides yet another source of antibody diversity (Fig. 4). For example, identical J_H segments may be associated with differing V_H segments (such as M104E and Hdex8; J558 and Hdex9). Conversely, identical V_H segments may be associated with different J_H segments (for example, Hdex1, Hdex2 and Hdex4) (Fig. 1). Even more striking is the observation that the J_1 segment in V_H regions from antibodies binding dextran also occurs in the very different V_H regions from myeloma proteins binding phosphorylcholine (Fig. 3). Likewise, the same J_H segment may be found in heavy chains binding phosphorylcholine and galactan (for example, M167 and T601). Thus, the combinational joining of J_H and V_H gene segments appears to be an important source of diversity in these dextran-binding antibodies.

D segment diversity. This most striking diversity pattern in these V_H regions (Figs 2, 3). Diversity in the D segments may arise from one of three general mechanisms: (1) a novel somatic mutation mechanism possibly occurring as a result of the joining of V_H and J_H gene segments; (2) a large number of germ-line J_H or V_H gene segments which include the D segment; or (3) a third gene segment (D) encoding positions 100 and 101 which is joined with the V_H and J_H gene segments during the differentiation of an antibody-producing cell. Let us consider what constraints the diversity patterns of the V_H regions impose on each of these models.

Several observations argue against many germ-line V_H gene segments extending from codons 1 to 101. First, identical D segments may be associated with different V_H segments—M104E-Hdex8 and J558-Hdex9 (Fig. 1). Thus, the D segments appear to associate independently with V_H segments. Second, the T15 V_H gene segment terminates at codon 99 (P. Early, H. Huang and L.H., unpublished observation). Accordingly, the D segment cannot be encoded as part of the V_H gene segment for the T15 V_H gene segment (Fig. 3). Identical D segments may be associated with different J_H segments—Hdex1-Hdex2 and X44-J539 (Fig. 3). A more striking example of this independent association of D and J_H segments is the observation that a J_1 segment is associated with very different D segments in immunoglobulins binding distinct haptens—T15 and M104E (Fig. 3). The independent segregation of D segments with both V_H and J_H segments suggests that the D coding regions are not merely germ-line extensions of V_H or J_H gene segments. In addition, homology comparisons among the V_H regions very clearly define the V_H and J_H segments (Fig. 3). In contrast, the D segments lack constancy both in size (0 to 7 residues) and amino acid sequence (20 different sequences in 25 different V_H regions compared) (Fig. 3). The V_H regions in Fig. 3 would require a very large number of germ-line gene segments if the V_H or J_H gene segments include the D segment.

The D segment diversity is quite distinct from the V–J junctional diversity of kappa chains in several regards. First, there are extensive size differences among the D segments whereas the κ junctional diversity shows at most the insertion or deletion of just a single codon[23]. Second, the two codons for the D segment associated with a particular $V_H(V_1)$ and a particular $J_H(J_1)$ segment exhibit three different nucleotide alternatives at three positions—100a, 100b and 101a (Table 1). This observation is important because it suggests that D segment diversity cannot be generated solely by recombination between the V_1 and J_1 gene segments as is suggested by the site-specific recombinational model of mouse kappa chains[21–23]. This model allows for only two alternatives at any nucleotide position. Accordingly, the extensive variability, the size differences and the presence of three bases at several nucleotide positions in the D segments constitute a distinctly new pattern of diversity that must be explained by a novel mechanism for somatic mutation or the presence of multiple D gene segments.

The striking base invariance at nucleotide positions 101b and

101c in the midst of the remarkable adjacent diversity raises several interesting possiblities (Table 1). Perhaps these invariant nucleotides form part of a recognition site for an enzyme that might mediate somatic mutation or join the D and J_H gene segments together. Alternatively, perhaps these invariant nucleotides are a part of the J_H gene segment.

If the D segment is encoded by a gene segment separate from the V_H and J_H gene segments, then heavy chain genes differ in a major organisational feature from light chain genes. Moreover, the presence of a putative D gene segment creates a second gene segment boundary (V_H–D and D–J_H) at which junctional diversity of the type noted in kappa chains can occur[21-23].

Antigen-binding diversity and V_H variability

The third hypervariable region forms an important part of the antigen-binding site[15,16]. Diversity in the dextran antibodies is concentrated in the third hypervariable region of heavy chains which includes the D segment and much of the J_H segment (Fig. 2). Studies on the binding specificites of the myeloma proteins M104E and J558 which differ only in the D segment have demonstrated that the antigen-binding properties of these two molecules are quite distinct[34,35]. Accordingly, the mechanism for producing D segment diversity does have an important role in modifying the antigen-binding properties of antibodies.

Variability in the third hypervariable region of the dextran antibodies arises from germ-line diversity (J_H segments), by combinational joining of V_H and J_H (and possibly D_H) segments and by the mechanism for producing D segment diversity. Of these, D segment diversity appears to be by far the most extensive and, presumably, important in generating a diversity of antigen-binding sites (Figs 2, 3).

One striking feature evident in Fig. 3 is that immunoglobulins binding particular haptens share similar V_H segments, exhibit D segments of similar size (for example, zero for levans, four for galactans, and so on), and to a lesser extent share similar J_H segments. Whether these correlations arise through some intrinsic properties of gene organisation, somatic mutational mechanisms or antigen selection is unknown.

Correlation of dextran idiotypes and protein structure

Several interesting conclusions can be drawn about the correlation of V_H structure and the various idiotypes of dextran-binding antibodies (Fig. 1). First, the individual idiotype appears to correlate with the D segment sequence. The V_H regions from the M104E and J558 proteins differ only by their D segments. As the light chains are identical, the individual idiotypes of M104E and J558 must be determined by positions 100 and 101. Moreover, the V_H region of Hdex9, which differs from that of J558 by four residues and is identical at positions 100 and 101, has the individual J558 idiotype. Likewise, the V_H region of Hdex8, which differs from that of M104E by seven residues and is identical in the D segment, also has the individual M104E idiotype. However, a single residue substitution can eliminate the individual idiotype as demonstrated by the observation that Hdex3 is lacking both the M104E and J558 individual idiotypes (Figs 1, 2). Partial and in one case complete IdI reactivity can be retained by D segments sharing one of two residues (Hdex1, Hdex2 and Hdex7). The overall conclusion is that the individual idiotypes of myeloma proteins M104E and J558 are determined by residues 100 and 101 and, accordingly, are either encoded by a third gene segment (D) or represent antigenic determinants generated by a novel somatic mutational mechanism. Second, the IdX idiotype correlates with several residues in the V_H segment. The V_H region from one IdX-negative protein, Hdex10, has been sequenced. Hdex10 differs from the two myeloma V_H regions by four residues—positions 54, 55, 100 and 101. The substitution at position 55 destroys the attachment site for the carbohydrate moiety, distinguishing it from the other

Protein	Specificity	V segment	D segment	J segment	References
		75 80 90 99	100 101	102 110 117	
M104E	1,3 Dextran	SSSTAYMQLNSLTSEDSAVYYCARD	YD	WYFDVWGAGTTVTVSS	32
Hdex8	"	---G---------------------	YD	----------------	This paper
J558	"	-------------------------	RY	----------------	"
Hdex9	"	--N--F-------------------	RY	----------------	"
Hdex10	"	-------------------------	VN	----------------	"
Hdex6	"	-------------------------	SH	----------------	"
Hdex3	"	-------------------------	R-	----------------	"
Hdex7	"	-------------------------	A-	----------------	"
Hdex2	"	-------------------------	NY	----------------	"
Hdex1	"	-------------------------	NY	H----V----------	"
Hdex5	"	-------------------------	SN	Y---Y--Q---L----	"
Hdex4	"	-------------------------	K-	Y---Y--Q---L----	"
T15,S63,S107,Y5236	Phosphorylcholine	-Q-IL-L-M-A-RA--T-I------	YYGSSY	---------------	46
H8	"	-Q-IL-L-M-A-RA--T-I------	---N--	----------------	"
W3207	"	-Q-IL-F-M-A-RA--T-I-----N	--KYDL	--V----------	"
M603	"	-Q-IL-L-M-A-RA--T-I-----N	----T	-------------	47
M167	"	-Q-VL-L-M-A-RA--T-T---T--	ADYGDSYF	G------------	48
M511	"	-Q-IL-L-M-A-RA--T-I------	GDYG-SY	---------------	"
A4	2,1 Levan	-K-SV-L-M-N-RA--TGI---TTG	[ ]	[ ]-AY--Q--L---	49
E109	"	-K-SVFL-M-N-RA--TGIH--TTG	[ ]	[ ]-AY--Q--L---	"
U61	"	-K-SV-L-M-N-RA--TGI---TTG	[ ]	[ ]-AY--Q--L-P-	"
A47N	"	-K-SV-L-M-N-RA--T-I---STG	[ ]	[ ]-AY--Q--L---	"
M315	DNP	-ENQFFLK-D-V-[ ]T-T----G-	NDH	L---Y--Q---L---	50
M21	Unknown	PKN-LFL-MT--R---T-M-----H	GNYPW	YAM-Y--Q--S-----	51
M173	Unknown	AKN-L-L-MSKVR---T-L-----S	PY	YAM-Y--Q--S-----	52
T601	Galactan	AKN-L-L-MSKVR---T-L-----G	GYY	G---------------	35
X44	"	AKN-L-L-MSKVR---T-L-----L	H--	G-AAY--Q--L----A	"
X24	"	AKN-L-L-MSKVR---T-L-----G	---	G---Y--Q---L----	"
J539	"	AKNSL-L-MSKVR---T-L-----L	H--	G-NAY--Q--L----A	"

Fig. 3 A comparison of published heavy chain sequences including the C-terminal portions of V_H regions. The division of this region into V_H segments, D segments, and J_H segments is based on homology with the heavy chains binding dextran. The prototype sequence for the V_H and J_H segments is M104E. The prototypes for the D segments are the first sequence in each group. The J segments which do not extend to amino acid 117 are due to incomplete amino acid sequence data and not to shorter J segments.

11 V_H regions (Fig. 1). It is attractive to postulate that the IdX idiotype depends on amino acids at positions 54 and 55. This supposition is supported by the observation that Hdex8, which has greatly reduced IdX reactivity, has a substitution at position 54. Analysis of V_H regions from several additional IdX-negative antibody molecules are in progress to determine whether they also exhibit substitutions at positions 54 and 55 and to test the intriguing possibility that the IdX idiotype may be in part associated with a carbohydrate moiety.

Thus, idiotypes can apparently arise from V segments (IdX) or D segments (IdI). These data on the hybridoma antibodies binding α-1,3 dextran do allow us to assign structural correlates for the cross-reactive (V segment) and individual (D segment) idiotypes of several dextran antibodies. However, an idiotype localised to a particular region (for example, the IdIJ558 to positions 100 and 101) may require particular sequences in still other portions of the V region for its expression (for example, V and J segments like those found in dextran-binding antibodies). Accordingly, the interpretation of genetic mapping of idiotypes is complex. Indeed, one can never be certain what is being mapped—a V segment, a D segment, a J segment, the ability to produce certain somatic variants, or some combination of these.

In an historical context, note that Kabat and coworkers predicted the existence of minigenes for each hypervariable region based on an analysis of the V region amino acid sequences[13,36]. In addition, Capra and Kindt postulated the existence of multiple V coding elements based on an analysis of V_H sequence data and idiotypes[37,38]. If the D segments are encoded by distinct gene segments, they are 'minigenes' that encode the individual idiotypes of murine antibodies binding dextran.

The evolution of split-gene segments and DNA rearrangements

The V_H regions and V_L regions are homologous to one another at the protein level[31,39]. Both V_L and V_H are encoded by V and J gene segments of similar size. This homology implies that the split-gene strategy and the mechanism for rearranging split genes must have arisen before the divergence of light and heavy chains. Indeed, it is attractive to speculate that split genes and mechanisms for their rearrangement evolved before the emergence of the vertebrate immune system, possibly in families of genes coding membrane recognition functions. It will be interesting to determine whether there are other contemporary multigene families using strategies of split genes and DNA rearrangment for the amplification of information[5]. This is discussed elsewhere[40].

The patterns of phenotypic diversity in the V_H regions from antibodies binding α-1,3 dextran have allowed us to make several important conclusions. We can define V_H and J_H segments which are homologous to their light chain counterparts. Diversity in a third region, the D segment, can be explained by a novel somatic mutational mechanism, the existence of a third germ-line (D) gene segment, or the less likely possibility of a large number of J_H gene segments. Antibody diversity appears to arise from several sources: multiple germ-line gene segments, combinational joining of V_H and J_H gene segments and the mechanism for producing D segment diversity which includes extensive nucleotide substitutions as well as the insertion or deletion of multiple residues. Correlations between the V_H sequences and the idiotypes of the antibodies to α-1,3 dextran are possible but there should be caution in the interpretation of genetic mapping studies which use idiotypes. Studies are in progress in our laboratories to expand our analyses of the phenotypic (protein) diversity in the dextran system and to analyse the gene segments encoding the dextran response.

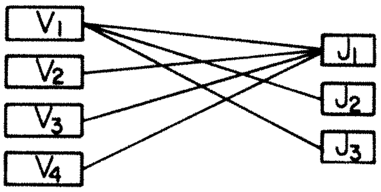


Fig. 4 A diagram indicating the combinational joining of V_H and J_H segments. V_1 to V_4 and J_1 to J_3 are defined in Fig. 2.

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A UGA termination suppression $tRNA^{Trp}$ active in rabbit reticulocytes

Alfred I. Geller & Alexander Rich

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

A $tRNA^{Trp}$ was purified from rabbit reticulocytes which suppresses the UGA termination codon of β-haemoglobin mRNA. Evidence is presented that the β-haemoglobin readthrough protein is found in reticulocyte translations and intact cells. Some natural readthrough proteins perform essential functions; they are synthesised through suppression of UGA or UAG but not UAA termination codons.

THE termination of polypeptide chain synthesis is signalled by the three codons UAG (amber), UAA (ochre) and UGA (opal). Termination is carried out through the activity of release factors which are thought to recognise termination codons and release the nascent polypeptide chain[1-3]. In some cases, termination is suppressed by a tRNA which recognises one of the termination codons and inserts an amino acid[4]. The ribosome then continues adding amino acids to the nascent chain until it encounters the next termination codon. Proteins which are synthesised in this manner are called 'readthrough' proteins and the tRNAs which recognise the termination codons are called suppressor tRNAs. Suppressor activity is of two types; naturally occurring and induced through mutagenesis. Natural suppression seems to be a mechanism for producing proteins which, while related to other proteins, nonetheless perform distinct and essential functions. Naturally occurring suppression has been largely associated with viral infection in prokaryotic as well as eukaryotic systems. The example of natural readthrough which has been studied in greatest detail is Qβ infection of *Escherichia coli*. The coat protein gene has a UGA termination codon. Wild-type *E. coli* $tRNA^{Trp}$ misreads the UGA codon at a low frequency, producing a coat readthrough protein[5-8] which is required for infection by Qβ[9]. An analogous situation may exist in bacteriophage λ infection of *E. coli* where the UGA termination codon of the O protein is read through[10,11]. Filamentous bacteriophages may also require UGA readthrough[11]. Recently, a readthrough protein produced by suppression of a UAG termination codon has been detected during the infection of tobacco plants by tobacco mosaic virus (TMV)[12]. However, this suppressor activity has not yet been associated with a particular tRNA.

Here we report a UGA suppressor activity in rabbit reticulocytes which produces a β-haemoglobin readthrough protein. A purified rabbit reticulocyte $tRNA^{Trp}$ dramatically enhances UGA suppression in the reticulocyte lysate. Furthermore, evidence is presented which strongly suggests that the UGA suppressor activity is active within intact reticulocyte cells producing very small amounts of the β-haemoglobin readthrough protein. This is the first demonstration of naturally occurring readthrough in a non-viral system.

Detection of suppression

Suppressor tRNAs from prokaryotic systems have been well characterised and sequenced. Although yeast suppressor tRNAs function in higher eukaryotic lysates[13,14] prokaryotic suppressor tRNAs often do not work in eukaryotic systems[13]. Our assumption was that this was due to the inability of eukaryotic aminoacyl tRNA synthetases to aminoacylate these tRNAs efficiently. Accordingly, we devised an *in vitro* system in which a purified prokaryotic tRNA and its purified cognate prokaryotic synthetase are added to a eukaryotic cell-free protein synthesising system. The well characterised rabbit reticulocyte system was used in which haemoglobin is the predominant (90–95%) product synthesised. Figure 1 shows reticulocyte translations using ^{35}S-methionine analysed on an SDS gel. Lane *a* shows the effect of adding the *E. coli* UGA suppressor without synthetase while lane *b* shows the effect of adding the UGA suppressor tRNA and *E. coli* Trp synthetase. A new band (*B*) appears which is somewhat larger than the two globin chains (Hb). Lane *c* shows the effect of adding an *E. coli* UAA suppressor tRNA and *E. coli* Tyr synthetase. A different protein (A) is produced which migrates more slowly than haemoglobin. Lane *d* shows the effect of adding the UAA suppressor tRNA without synthetase. Lane *e* is a control with no additions. The readthrough proteins A and B have distinct mobilities even when mixed together (data not shown).

α-Haemoglobin mRNA terminates in a UAA codon and the next termination codon in phase occurs 21 codons downstream[15]. This suggests that band A seen in Fig. 1 is the α-haemoglobin readthrough protein. Rabbit β-haemoglobin mRNA terminates in a UGA termination codon. The next termination signals, two UAAs in tandem, occur 22 codons later[16]. This suggests that band B in Fig. 1 is the β-haemoglobin readthrough protein. α- And β-haemoglobin readthrough proteins have also been synthesised when the appropriate yeast suppressor tRNAs are added to a wheat-germ extract[17]. In further experiments not shown here, an *E. coli* UAG suppressor tRNA and its purified tyrosine synthetase were added to the reticulocyte lysate but no readthrough bands were detected. The UAG suppressor was shown to function in the reticulocyte lysate using an RNA from a Qβ mutant (*am* − 1) which has a UAG nonsense mutation in the synthetase gene[14].

We observed faint bands in all of the lanes of Fig. 1 at the same position as the putative β-haemoglobin readthrough protein. This suggested that there might be natural readthrough of the UGA termination codon of rabbit β-haemoglobin mRNA. Before proceeding further, we carried out additional analysis of the readthrough band to demonstrate that it is in fact the β-haemoglobin readthrough protein. α- And β-haemoglobin chains and the β-haemoglobin readthrough protein were purified then subjected to tryptic digestion[18]. The results are shown in Fig. 2 and a diagram shows the position of the methionine-containing tryptic peptides in both α- and β-haemoglobin and their readthrough proteins[15,16]. α-Haemoglobin has one methionine-containing tryptic peptide composed of 9 amino acids. There are no additional methionines in its readthrough protein. β-haemoglobin has one methionine-containing tryptic peptide composed of 19 amino acids. That peptide, which ends in lysine, is followed by the sequence

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Val-Lys at its C terminus; in the case of incomplete cleavage at the first lysine residue, one may get a peptide containing 21 amino acids. The readthrough protein of β-haemoglobin has an additional methionine-containing tryptic peptide, 7 amino acids in length[16].

The tryptic peptides were analysed by electrophoresis at *p*H 3.5. Lane *a* of Fig. 2 has ^{35}S-methionine alone, lane *b* contains the tryptic digest of β-haemoglobin. The direction of migration is to the left (towards negative) and two spots are seen. This suggests that the slower spot contains 19 amino acids while the faster spot contains 21 amino acids, resulting from incomplete cleavage at the C-terminal lysine. Incomplete cleavage results in the addition of Val-Lys to the peptide and a doubling of its positive charge at *p*H 3.5. This assignment is strengthened by the observation that longer digestion periods yield more of the slower spot. The tryptic digest of β-haemoglobin was also analysed by chromatography[18]. The results of this experiment (not shown) yielded two spots which migrated at positions very close to each other, suggesting the general properties of these two peptides, including both length and chemical characteristics, are very similar. This is consistent with our identification of the second minor peptide as arising from incomplete digestion by trypsin. Lane *c* shows the tryptic digest of α-haemoglobin which yields one major spot. Lane *f*, a mixture of tryptic digests of α- and β-haemoglobin show three spots. The tryptic peptides of the putative β-haemoglobin readthrough protein are shown in lane *d*. This produced three spots, two of which migrated at the same position as the β-haemoglobin spots and one additional spot migrating between the two. Lane *e* shows a mixture of the tryptic digests of both α- and β-haemoglobin and the β-haemoglobin readthrough protein. Four spots are now observed. The conclusion from this experiment is that band B in Fig. 1 is indeed the β-haemoglobin readthrough protein as shown by the appearance of an additional methionine containing tryptic peptide which is expected in view of the mRNA sequence after the UGA termination codon.

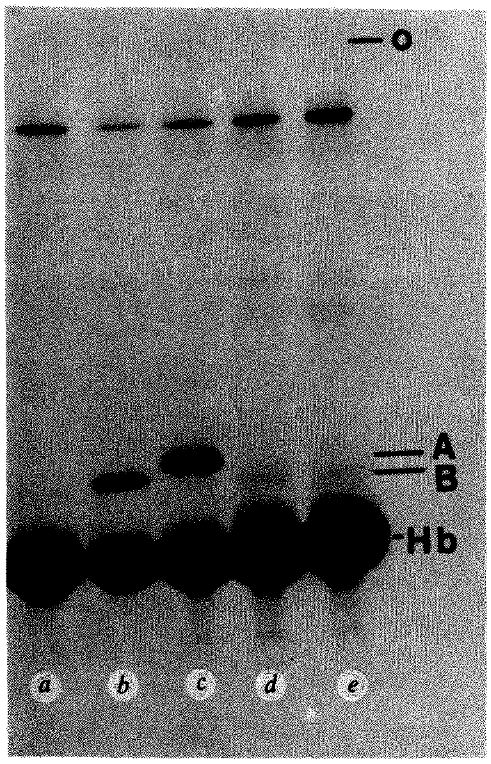

Fig. 1 Autoradiogram of rabbit reticulocyte lysates incubated with ^{35}S-methionine and analysed on an SDS gel. *E. coli* suppressor tRNAs were isolated from *E. coli* DB 6437 Sup C (UAA)[33] and LS 849 Su^{7+} (UGA)[34] (provided by David Botstein). LS 849 was grown as described in ref. 35. An S-30 supernatant was prepared[36] and then centrifuged at 100,000*g* for 5 h. The supernatant was extracted with phenol saturated with 10 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$, 30 mM NH_4Cl. After deacylation[37] and exhaustive dialysis against 200 mM NaAc (*p*H 5.0), the tRNA$^{\mathrm{Tyr}}$ (ref. 38) and tRNA$^{\mathrm{Trp}}$ (refs 23, 24) were purified and stored at −60 °C. *E. coli* Tyr aminoacyl tRNA synthetase[39] (Tyr R.S.) and *E. coli* Trp aminoacyl tRNA synthetase[23] (Trp R.S.) were gifts from P. Schimmel and K. Muench respectively. One part haemin and 0.4 parts creatine phosphokinase[40] were added to 100 parts reticulocyte lysate[41]. Reticulocyte translation solution (25 μl) contained 0.5 mM spermidine, 8 mM creatine phosphate, 25 μM amino acids except methionine, 2 mM dithiothreitol (DTT), 20 mM HEPES (*p*H 7.6), 0.65 mM $MgAc_2$, 80 mM KAc, 5 μl (5 mCi ml^{-1}) ^{35}S-methionine (Amersham), and 10 μl reticulocyte lysate. 5 μg tRNA and 0.2 μg aminoacyl tRNA synthetase were added as indicated below. Reaction mixtures were incubated at 37 °C for 1 h and stored at −20 °C for subsequent use. Reaction mixtures (1 μl) contained approximately 100,000 trichloroacetic acid (TCA) precipitable counts. Aliquots of 2 μl were loaded into each lane of a 15–20% linear gradient polyacrylamide SDS gel[42]. The gel was run for 1 h after the bromophenol blue (BPB) tracking dye ran off the bottom. The gel was stained, destained, fluorographed[43] and then exposed to XR-5 film for 1 d. Lane *a*, Su^{7+} (UGA) tRNA; lane *b*, Su^{7+} (UGA) tRNA and *E. coli* Trp R.S.; lane *c*, Sup C (UAA) and *E. coli* Tyr R.S.; lane *d*, Sup C (UAA) tRNA; lane *e*, no additions. The origin is marked O.

UGA suppression in reticulocyte lysate

Experiments were carried out to detect natural readthrough producing the β-haemoglobin readthrough protein. Two-dimensional gel electrophoresis[19] was used to separate the various products made by the reticulocyte lysate. Figure 3*a* shows a 12-hour exposure of a two-dimensional gel of a translation with *E. coli* UGA suppressor tRNA (hereafter all translation with *E. coli* suppressor tRNA also contained the appropriate *E. coli* synthetase). The small spot indicated by the vertical arrow is the β-haemoglobin readthrough protein while the larger spots below it are haemoglobin chains. Figure 3*b* shows a 1-week exposure of the same gel. Figure 3*C* is a 1-week exposure of a two-dimensional gel of a translation with no additions. A faint spot appears at the same position as the β-haemoglobin readthrough protein. This gel also revealed a few proteins with the same molecular weight as the β-haemoglobin readthrough protein but very different isoelectric points. These proteins prevented the purification of enough natural β-haemoglobin readthrough protein to permit tryptic analysis.

To ensure identification of this weak spot another electrophoretic experiment was carried out; a two-dimensional gel was run using isoelectric focusing in the first dimension and an acid-urea gel in the second[20]. The β-haemoglobin readthrough protein was detected by autoradiography. The gel fragment containing this spot was excised, rehydrated, and loaded on to an SDS gel for further analysis. A gel slice containing haemoglobin was excised from an SDS gel to run in the system as a marker. The control in Fig. 3*D*, lane *a*, contains two bands, one of which is haemoglobin. Since all the protein in lane *a* is haemoglobin, the second band is likely to be a dimer of two haemoglobin chains. Crosslinking could have occured during either staining, destaining, drying or autoradiography. Lane *b* came from a translation containing the *E. coli* UGA suppressor analysed as described above; in addition to haemoglobin and haemoglobin dimers, it contains the β-haemoglobin readthrough protein labelled B. Lane *c* was a translation with no additions analysed on a two-dimensional gel. This lane also shows a weak band which co-migrates with the β-haemoglobin readthrough protein. This evidence strongly suggests that there is a low level of natural readthrough of the UGA termination codon of β-haemoglobin mRNA in the rabbit reticulocyte lysate.

Readthrough proteins in rabbit reticulocyte cells

An experiment was carried out to detect synthesis of the β-haemoglobin readthrough protein in intact rabbit reticulocyte cells. Reticulocytes were incubated in a cell culture medium in the presence of radioactive amino acids[21]. The proteins were separated on two-dimensional gels[19]. Figure 4*a* shows an analysis of the mixture of the incubation and a translation with

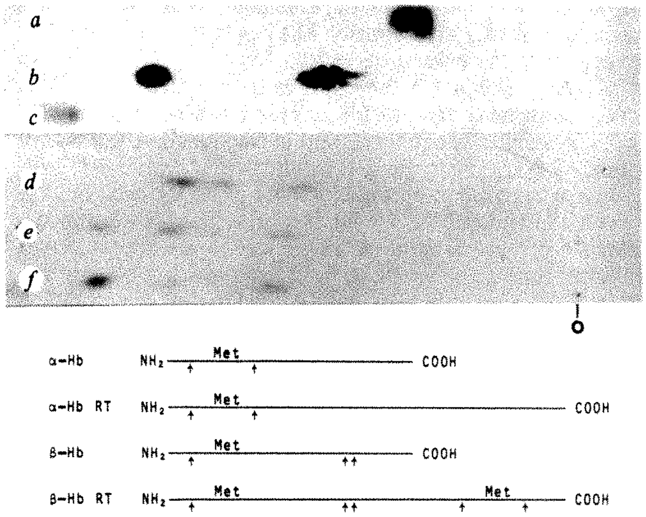

Fig. 2 Autoradiogram of tryptic digestion of various haemoglobin chains. At the bottom is a diagram showing the α- and β-haemoglobin (Hb) chains as well as their readthrough (RT) proteins. The vertical arrows indicate the positions of tryptic digestion sites around methionine-containing peptides. A 25-μl translation mixture was run on a 15% polyacrylamide SDS phosphate gel[44] for 20 h at 80 mA to separate ^{35}S-methionine-labelled α- and β-haemoglobin. A 25-μl translation mixture with Su^{7+} (UGA) tRNA and *E. coli* Trp R.S. was run on an SDS gel[42] as in Fig. 1 to purify the putative β-haemoglobin readthrough protein. Gels were autoradiographed to locate the protein. Samples were prepared and digested with trypsin as described[18] with the following modifications; 4 μl of 25 mg ml^{-1} bovine serum albumin was added to the β-haemoglobin readthrough protein sample; the α- and β-haemoglobin samples required no carrier. After the final lyophilisation, the samples were taken up in 20 μl H_2O. The tryptic peptides were analysed by electrophoresis on cellulose-coated thin layer plates for 90 min at 1,000 V, 25 mA in pyridine : acetic acid : H_2O, 1 : 10 : 100 (*p*H 3.5). The plate was immersed in PPO-β-napthalene[45] and then exposed to film for 2 d except for lane *c* which was exposed for 1 d. Lane *a*, 3,000 c.p.m. ^{35}S-methionine. The following tryptic digests: lane *b*, 3,000 c.p.m. β-haemoglobin; lane *c*, 3,000 c.p.m. α-haemoglobin; lane *d*, 1,500 c.p.m. β-haemoglobin readthrough protein; lane *e*, 500 c.p.m. α-haemoglobin and 500 c.p.m. β-haemoglobin and 500 c.p.m. β-haemoglobin readthrough protein; lane *f*, 750 c.p.m. α-haemoglobin and 750 c.p.m. β-haemoglobin. The origin is marked O.

an *E. coli* UGA suppressor; the 1-week exposure shows a well developed spot in the position of the β-haemoglobin readthrough protein as indicated by the arrow. Figure 4*b* is a 1-month exposure of just the incubation of intact cells; several additional spots appeared after the longer exposure. There is a small spot which migrates at the position of the β-haemoglobin readthrough protein. Because of the low level of the putative β readthrough protein in intact cells, no further analysis was attempted. It seems likely that there is a low level of production of the β-haemoglobin readthrough protein in intact reticulocyte cells.

tRNA fractionation and suppressor activity

The results presented above suggested that there might be a tRNA in the reticulocyte cell which suppresses the UGA termination codon. Reticulocyte tRNA was fractionated on BD cellulose[22], widely used in tRNA purification, to purify this activity. As shown in the graph at the top of Fig. 5, the bulk of the tRNA emerges in the first third of the column. The assay shown for tryptophan acceptor activity has a maximum near fraction 13, and a leading shoulder in fractions 5–8. Each of these fractions was precipitated and then redissolved in H_2O. A sample of 4 μg of tRNA from each fraction was added to a 25 μl reticulocyte translation representing a 10–20% increase in the amount of tRNA in the reaction. The results are shown at the bottom of Fig. 5. The lanes marked Su$^+$ and Su$^-$ were controls with the *E. coli* UGA suppressor added and with no additions, respectively. The Su$^+$ lane shows the β-haemoglobin readthrough protein which is enhanced in fractions 5–8. In fraction 6 a number of other bands are also enhanced for an unknown

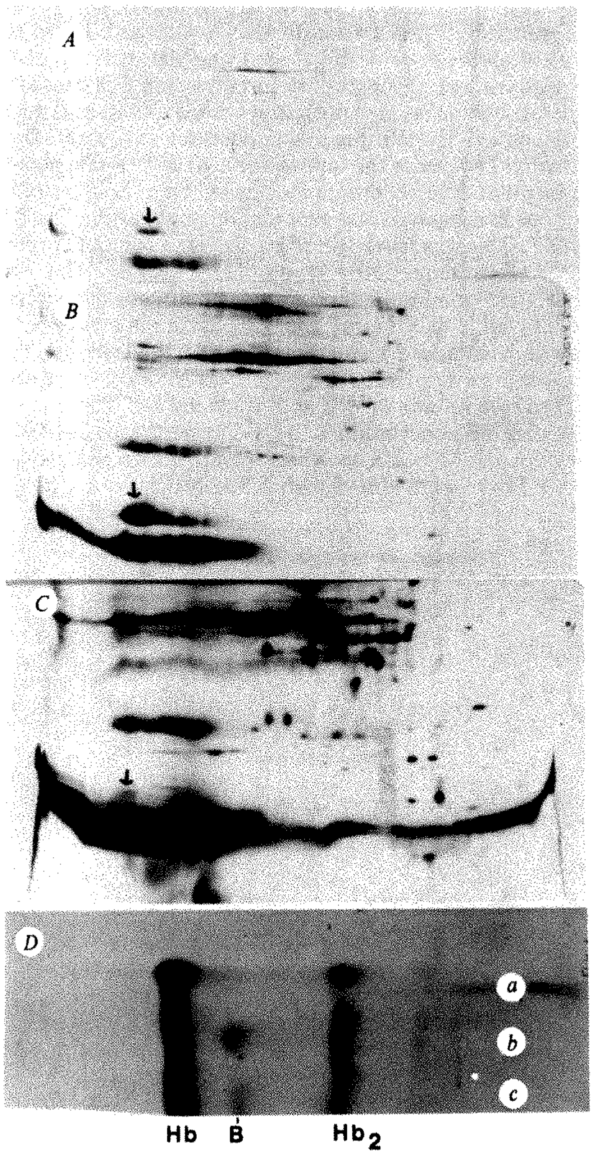

Fig. 3 Autoradiograms of ^{35}S-methionine-labelled reticulocyte translation mixtures analysed in various gel systems. Two-dimensional gels[19] were run with *p*H 6–8 and *p*H 3–10 ampholytes in the ratio of 4:1 in isoelectric focusing. The second dimension (vertical) was a 15–20% linear gradient polyacrylamide SDS gel. The gel was treated as in Fig. 1. *a*, 5×10^5 c.p.m. of a translation mixture with Su^{7+} (UGA) tRNA and *E. coli* Trp R.S. The film was exposed for 12 h. *b*, 1-week exposure of gel *a*. *c*, 1.5×10^6 c.p.m. of a translation mixture with no additions. The film was exposed for 1 week. *d*, Readthrough spots were isolated from isoelectric focusing acid-urea two-dimensional gels and run on SDS gels. Isoelectric focusing tube gels were run as above[19]. The gels were equilibrated in buffer O′ (5% HAc, 10% glycerol) for 30 min and then stored at −60 °C or used immediately. Slab gels of 15% polyacrylamide acid-urea[20] were pre-run for 1 h at 125 V. The equilibrated tube gels were loaded on top of acid-urea gels and overlaid with freshly made 1% agarose in buffer O′ in the same manner as the second dimension of two-dimensional O'Farrel gels[19]. Gels were run for 20 h at 125 V, destained, dried and autoradiographed for 1 week. The appropriate gel cubes were located, excised, rehydrated in buffer O[19] for 30 min, and then analysed on an SDS gel[42] as in Fig. 1. Only the SDS gel is shown: lane *a*, the haemoglobin band from a translation mixture was isolated from an SDS gel run as in Fig. 1. The gel slice was rehydrated in buffer O and loaded on to the SDS gel. The control haemoglobin now appears as two spots, probably due to dimerisation. Lane *b*, the β-haemoglobin readthrough spot from 5 μl of a translation mixture with Su^{7+} (UGA) tRNA and *E. coli* Trp R.S. was isolated from an isoelectric focusing acid-urea two-dimensional gel. Lane *c*, 25 μl of a translation mixture with no additions was assayed in a way analogous to *b*. The gel was exposed to film for 2 months.

reason. These bands are all detected in reticulocyte translations with no additions on longer exposures; the bands are resistant to RNase digestion (results not shown) so they are not polypeptide chains prematurely released with tRNA attached. From this experiment alone, it could be argued that the increase of the β-haemoglobin readthrough protein in fraction 6 is caused by the general enhancement of protein synthesis activity and not by a suppressor activity. We therefore undertook to separate these two activities and purify the suppressor activity to homogeneity.

The fact that the enhancement occurred on a shoulder of the $tRNA^{Trp}$ peak suggested that suppression might be mediated by a $tRNA^{Trp}$. This was investigated by a second column chromatography of aminoacylated tryptophanyl-$tRNA^{Trp}$ on BD-cellulose. BD-cellulose has a strong affinity for planar ring molecules and binds reticulocyte tryptophanyl-$tRNA^{Trp}$ because of the attached aromatic amino acid; this is the standard method for purifying *E. coli* $tRNA^{Trp}$ (refs 23, 24). As shown in Fig. 6, the column was loaded with fractions 5–8 from the first column after aminoacylation with ^{3}H-Trp. Fractions 3–13 were eluted with 0.8 M NaCl to wash off all uncharged tRNAs. Fractions 14–18 were eluted with 1.5 M NaCl, 20% EtOH to wash all tRNA off the column. The final wash resulted in a significant peak of optical density. The peak of ^{3}H-Trp-$tRNA^{Trp}$ emerged from the column largely in fractions 14 and 15. No Trp-$tRNA^{Trp}$ eluted off the column before that point. The fractions were also assayed for tryptophan-accepting activity, (Fig. 6). A small amount was found in fractions 1–13, however fractions 14–17, comprising the final wash, contained large amounts of tryptophan-acceptor activity. Since the ester linkage between Trp and $tRNA^{Trp}$ is labile with a half life of approximately 6 h in these conditions, a significant amount of $tRNA^{Trp}$ is released during the purification process which took approximately 10 h. Almost half the $tRNA^{Trp}$ came off with the final wash.

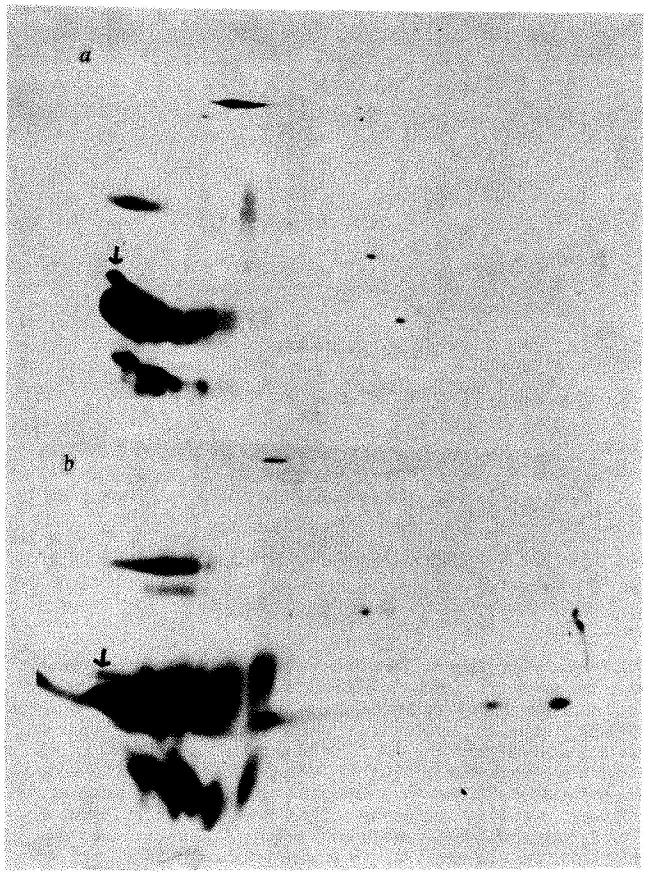

Fig. 4 Autoradiographs of two-dimensional gels analysing labelled reticulocyte proteins. Reticulocytes were incubated[21] with the following modifications: the 10-ml incubation mixture contained Krebs–Ringer phosphate, 0.001% heparin, 2 ml dialysed calf serum, 0.2 mM cysteine, 2 mM glutamine, 0.05 mM tryptophan, 0.2 mM asparagine, 10 mM 2-mercaptoethanol (2-ME), 1 mg ml^{-1} glucose, 1.8 mCi ^{35}S-methionine and 250 μCi of a ^{3}H-labelled amino acid mixture (Amersham). After the incubation, the cells were centrifuged at 15,000g for 5 min, washed once in Krebs–Ringer phosphate, centrifuged, lysed in an equal volume of H_2O containing 10 mM phenylmethylsulphonyl fluoride and then centrifuged for 20 min to remove cell membranes. The supernatant was precipitated with 10% TCA for 30 min at 0 °C and centrifuged for 10 min. The pellet was redissolved in one volume of 10% glycerol, 10% SDS, 2 mm EDTA, 1 M DTT, 100 mM Tris-HCl (*p*H 7.5), 0.1% BPB and titrated to *p*H 7.0 with 3 M NaOH. The sample was boiled for 5 min and stored at −20 °C for subsequent use. Two-dimensional gels[18] were run as in Fig. 3. *a*, 7.2×10^4 c.p.m. of the intact cell incubation plus 2×10^5 c.p.m. of an *in vitro* translation mixture with Su^{7+} (UGA) tRNA and *E. coli* Trp R.S. The film was exposed to the gel for 1 week. *b*, 2×10^5 c.p.m. of the intact cell incubation. The film was exposed to the gel for 1 month.

The autoradiogram shown in Fig. 6 shows the effect of these various fractions on translation in the reticulocyte system. There is a small enhancement of readthrough in many of fractions 1 to 15. However, a very large increase in readthrough is observed in fractions 16 and 17. Interesting, in fractions 14–17, there is high tryptophan-acceptor activity. Figure 5 shows the reticulocyte suppressor $tRNA^{Trp}$ and the nonsuppressing $tRNA^{Trp}$ were incompletely separated; it is likely that the second column achieved a better separation. The total radioactivity incorporated in translations of fractions 16 and 17 in Fig. 6 was somewhat less than that found in translations containing other fractions. This is probably associated with a higher amount of salt in that fraction which was not fully washed out. Longer exposure of the autoradiogram in Fig. 6 revealed that the general stimulatory activity migrated with the major peak of tRNA, fractions 2–5. A densitometer tracing of the optical density in the β-haemoglobin readthrough protein band compared to the haemoglobin band indicated that the intensity of the bands is approximately equal. The haemoglobin band contains a mixture of both β and α chains, while the readthrough band contains only β-haemoglobin readthrough protein. This suggests that a very high efficiency of readthrough is produced by fractions 16 and 17. It appears more efficient than the *E. coli* UGA suppressor (lane Su$^+$). Electrophoresis on two-dimensional gels showed that the readthrough protein synthesised in translations containing fractions 16 and 17 comigrated with the β-haemoglobin readthrough protein and migrated differently from the α-haemoglobin readthrough protein in both dimensions (results not shown).

Discussion

Analysis of translations with no additions revealed a small amount of a peptide which had the identical mobility as the β-haemoglobin readthrough protein in three different electrophoretic systems. This strongly suggested the presence of a UGA suppressor tRNA in the reticulocyte. This was examined by fractionating reticulocyte tRNA and isolating a peak which enhanced the production of the β-haemoglobin readthrough protein. This was identified as a $tRNA^{Trp}$ by separating tryptophanyl-$tRNA^{Trp}$ from all other tRNAs. Proteins from intact rabbit reticulocyte cells incubated with ^{35}S-methionine were analysed by two-dimensional gel electrophoresis. A small spot was detected in the same position as authentic β-haemoglobin readthrough protein. Although this identification does not establish proof, coupled with the isolation of a tRNA from the reticulocyte cell which reads the UGA termination codon, this result strongly suggests that we have detected a low level of β-haemoglobin readthrough protein in the intact reticulocyte cell.

The β-haemoglobin readthrough protein appears to be stable in *in vitro* incubations. Additional incubation in the presence of glucose and/or ATP did not degrade the β-haemoglobin readthrough protein nor did its position change in two-dimensional gel electrophoresis which, for example, might occur as a result of glycosylation. The readthrough activity in the TMV[12] and Qβ[25] systems are sensitive to changes in Mg^{2+} concentration. The concentrations of Mg^{2+}, K^+ or Na^+ have little influence on the amount of β-haemoglobin readthrough protein produced (data not shown). The material which produces the readthrough is likely to be RNA as KOH treatment abolishes production of the β-haemoglobin readthrough protein. The position of this RNA molecule on a column changes after it has been aminoacylated with tryptophan. It is on this basis that we feel confident that there is a reticulocyte $tRNA^{Trp}$ which reads the UGA termination codon. It has also been shown that $tRNA^{Trp}$ reads UGA in other systems[5-8,26].

The tRNA species which has been purified is highly active in producing the β-haemoglobin readthrough protein; it is not likely that it is operating by making occasional mistakes. This tRNA is now being purified further with a view to determining its complete nucleotide sequence. This will allow us to determine whether it is reading UGA because of a mutation in the anticodon which makes it complementary to UGA or because of a difference in nucleotide modification from the more abundant reticulocyte nonsuppressing $tRNA^{Trp}$. Methylation and other nucleotide modifications have been shown to alter the reading abilities of other tRNAs[4]. Misreading in this case requires an A · C base pair, therefore it cannot be described as wobble misreading.

Broadly considered, there are two different types of termination suppression. One is induced suppression, produced by mutagenesis, which has been studied extensively in prokaryotic and lower eukaryotic systems. A nonsense mutation produces a termination codon in the middle of the structural protein and a second mutation produces a termination suppressor tRNA which reads the termination codon restoring the original polypeptide. The second class are natural suppressors; these occur in the wild-type organism. These have not been studied extensively and our knowledge is relatively meagre. The best characterised example of naturally occurring suppression is the coat readthrough protein which is synthesised during infection of *E. coli* cells by Qβ virus; wild-type *E. coli* $tRNA^{Trp}$ suppresses the UGA termination codon of the coat protein[5-8]. The readthrough protein, produced at low frequency, is essential for the production of infective Qβ virions[9]. The function of the readthrough protein is not known. Another example of naturally occurring UGA suppression is found in bacteriophage λ infection of *E. coli* where the O protein is read through producing the readthrough protein O′ (refs 10, 11). As in Qβ infection, the O′ protein appears to be essential for infection[11]. Very little is known about natural readthrough in eukaryotic systems. A UAG (amber) suppressor tRNA may be used in the translation of a tobacco mosaic virus mRNA[12]. Addition of yeast amber suppressor tRNA to the reticulocyte system enhances the production of a 160,000 MW protein and diminishes a 110,000 MW protein. These proteins both have the same N-terminal sequence and have been isolated from the infected plant leaves. The 160,000 MW protein is produced in the rabbit reticulocyte lysate suggesting that it has a natural UAG suppressor activity. A UAG suppressor activity has also been inferred in the production of the polymerase protein in murine leukaemia virus infection[27,28] but appears not to be used in the related Rous sarcoma virus infection[29].

The UGA suppressor activity we have demonstrated in the reticulocyte is the first example of a natural suppressor activity not associated with viral infection. It suggests that naturally occurring termination suppressors may be active in non-viral systems. Further analysis will be necessary to determine the extent to which these naturally occurring suppressors are found in other systems. Preliminary results from experiments involving the addition of fractionated yeast tRNA to the reticulocyte lysate and translations of rabbit reticulocyte mRNA in the wheat-germ system indicate a UGA suppressor activity in both these sources.

The existence of haemoglobin molecules with C-terminal additions is not unknown. In humans, mutations are found in the termination codons of both α- and β-haemoglobin mRNA (haemoglobins Constant Springs and Tak[30]). Apparently, the addition of a peptide at the C terminus does not grossly interfere with physiological function although not much is known about other effects it may have.

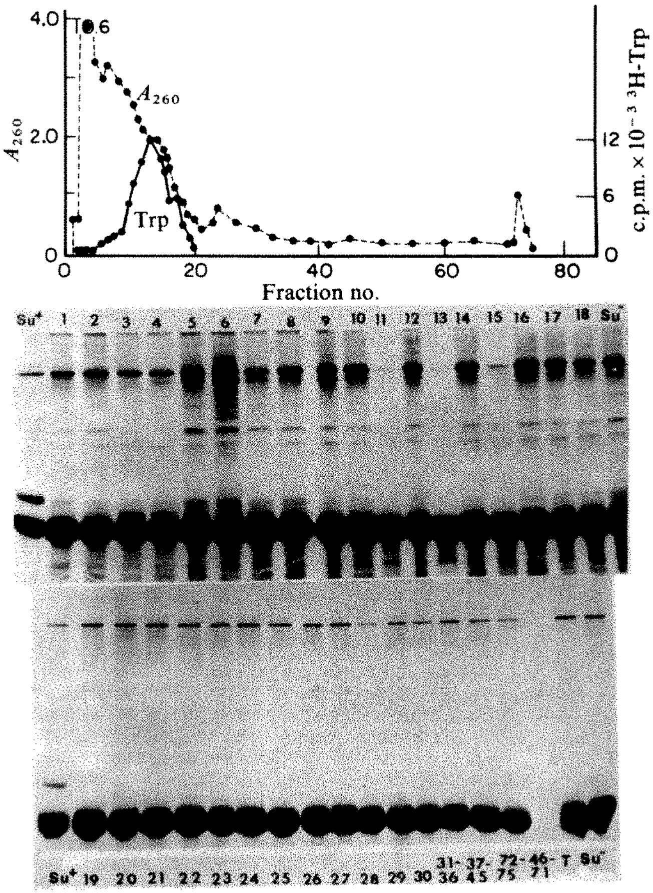

Fig. 5 Fractionation of reticulocyte tRNA on BD-cellulose and an assay for the production of readthrough proteins. Reticulocyte lysate[41] was centrifuged at 100,000*g* for 5 h. The supernatant was extracted with phenol saturated with 0.15 M NaCl, 10 mM Tris HCl (*p*H 7.5), 1 mM EDTA, then deacylated[37] and dialysed overnight against 200 mM NaAc (*p*H 5.0). The RNA was loaded on to a 12-ml BD-cellulose column[22] in 200 mM NaCl and developed with a linear 500 ml gradient from 0.4 to 0.9 M NaCl (fractions 1 to 71). This was followed by a 10% EtOH, 1 M NaCl wash (fractions 72 to 75). All column buffers contained 25 mM Tris-HCl (*p*H 7.5), 10 mM $MgCl_2$. Yeast Trp R.S. was isolated: 1 lb of baker's yeast was lysed with toluene[46] and then centrifuged at 13,000*g* for 40 min. The supernatant was made 100 mM Tris-HCl (*p*H 7.5), 30 mM 2-ME, 10 mM $MgCl_2$ and the *p*H was adjusted to 7.5 with 2 M NaOH. 0.1 volume of 1 M BaCl was added and the solution was centrifuged for 30 min. The supernatant was made 20 mg ml^{-1} in streptomycin sulphate and centrifuged for 30 min. The supernatant was precipitated with $(NH_4)_2SO_4$ (ref. 46) and the pellet redissolved with and dialysed against 20 mM $KHPO_4$ (*p*H 7.0), 1 mM DTT and stored in 50% glycerol at −20 °C. Charging assays were performed as described in ref. 46 on 8 μl of each fraction for Leu, Tyr, Lys, Gly and Trp. Only the Trp acceptance curve is shown. Fractions were precipitated, washed twice with 66% EtOH, taken to near dryness under a vacuum, and redissolved in H_2O to a concentration of 2 μg μl^{-1}. 2 μl were added to a 25-μl reticulocyte translation mixture which was incubated and analysed as in Fig. 1. The lane numbers refer to translations of the various fractions. The Su^+ lane contained Su^{7+} (UGA) tRNA and *E. coli* Trp R.S., the Su^- lane had no additions. Lane 46–71 was inhibited by excess salt. Lane T contained a reaction with unfractionated reticulocyte tRNA. The top gel was exposed to film for 4 d and the bottom gel for 3 d.

A review of the literature on termination codons and natural suppressor tRNAs suggests that there are significant differences between the three termination codons. The UAA (ochre) codon has no known naturally occurring suppressor tRNAs. In the haemoglobin system, we have an assay for the presence of UAA readthrough but no activity is found in reticulocyte tRNA. Induced UAA suppressor tRNAs in prokaryotic systems are found to be detrimental causing a doubling of the generation time[4]. It is possible that efficient UAA suppressors might be lethal. However, cells with either UGA or UAG suppressor tRNAs appears to be healthy and indeed some readthrough proteins made by suppressing these two termination codons seem to be used in natural systems.

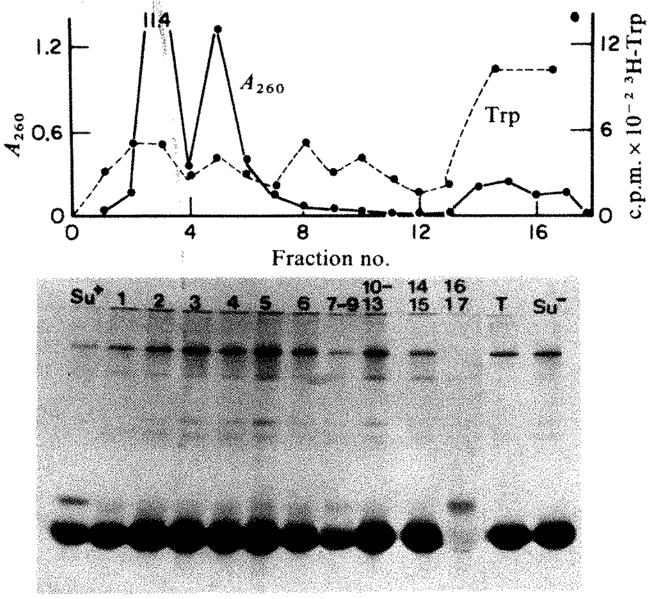

Fig. 6 Further fractionation of fractions 5 to 8 of Fig. 5 after aminoacylation with tryptophan. Yeast synthetase was passed over a G-100 column to remove any remaining yeast tRNA. Fractions 5 to 8 from Fig. 5 were charged with ^{3}H-Trp[46]. The reaction was stopped with the addition of 1/10 volume 2 M NaAc (*p*H 5.0) and 1 volume phenol saturated with 50 mM NaAc (*p*H 5.0). All operations were at 4 °C. The tRNA was precipitated, washed once with 66% EtOH and redissolved in 2 ml 0.4 M NaCl and loaded on to a 12-ml BD column[22]. The first half of fraction 1 represents the effluent from loading the sample. The last half of fraction 1 and fraction 2 were eluted with 0.4 M NaCl. Fractions 3 to 13 were eluted with 0.8 M NaCl and fractions 14 to 18 were eluted with 1.5 M NaCl, 20% EtOH. All column buffers contained 10 mM $MgCl_2$ and 50 mM NaAc (*p*H 5.0). 50 µl of each fraction was TCA precipitated. The ^{3}H-Trp tRNA^{Trp} peak (not shown) was primarily in fraction 14 but also in fractions 15 to 17. None was detected before fraction 14. 8 µl of each fraction was assayed for Trp acceptance activity[46] as shown in the graph. Fractions 14 –15 and 16–17 were pooled before the assay. The fractions were then assayed for suppression activity as in Fig. 5. The film was exposed for 2 d.

Evolutionary implications of termination suppression

The difference in the way nature uses termination codons suggests a rationale for the existence of three termination codons in the genetic code. It is possible that termination codons have different meanings. Perhaps the codon UAA means that translation must 'always stop' and perhaps there are very few (or no) exceptions to this in normal cells. The codons UAG and UGA may mean 'usually stop'. However, naturally occurring tRNAs will occasionally read UAG or UGA, resulting in the production of natural readthrough proteins. In addition, double stop signals are used to ensure the complete termination of protein synthesis after a given codon and are abundant in prokaryotic systems[31]. A low level of natural suppressor would be unable to read through a double stop system at a significant frequency. The selective utilisation in mRNAs of the three different termination codons and multiple stop signals allows the systems to have complete stop signals as well as partial or controllable stop signals. Nature has thus achieved a measure of control of gene expression at the level of termination. Diversification and evolutionary experimentation can then be carried out; occasionally a peptide is added to the C terminus of a selected protein. The resulting readthrough protein may perform a unique and perhaps essential function.

We suggest that natural readthrough is used occasionally to create proteins in which two different polypeptide chains are joined together. This ability may be used in a selective fashion, perhaps to control key biological processes. It would be of interest, for example, to see whether suppressor tRNAs are synthesised at key points in cellular development or perhaps during oncogenic conversion. Development of this process has provided an opportunity for exploiting the effects of producing novel proteins by joining different segments.

Is it possible that this type of experimentation was found to be very useful in the evolution of biological systems? Perhaps natural readthrough was a precursor to a more complex system of synthesising proteins by bringing together different polypeptides: RNA splicing[32]. The power of the splicing system, in contrast to the readthrough system, is that the segments to be joined together need not be contiguous in the genome of the organism. Splicing is also far more efficient than readthrough. Nonetheless, the net result of splicing and natural readthrough may be quite similar. We suggest that the utility of using occasional readthrough proteins which join together different polypeptide segments may have some of the positive features which are present in the more sophisticated RNA splicing mechanism which plays a significant role in the expression of genetic information in eukaryotic cells.

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LETTERS

Superclusters and galaxy formation

Jaan Einasto, Mihkel Jôeveer & Enn Saar

Tartu Astrophysical Observatory, Tôravere 202444, Estonia, USSR

Most galaxies and rich clusters of galaxies[1] are concentrated in superclusters, and there are great distances between superclusters[2–6]. In this paper we study the structure of superclusters using Zwicky clusters[7] as principal tracers of the large-scale structure of the Universe. Our study is restricted to the southern galactic hemisphere.

Using Huchra's compilation of available redshifts of galaxies we have found mean redshifts of Zwicky clusters of distance class 'near' in the region 22 h $\le \alpha \le$ 4 h. Of the 88 clusters in the area considered, redshifts have been measured for 56. The redshifts of the remaining 32 clusters have been estimated on the basis of luminosity function and apparent diameter. The distribution of clusters in the sky in two redshifts intervals is given in Fig. 1.

The prominent triangle-shaped aggregate seen in Fig. 1*a* is the Perseus supercluster. Its richest cluster A426[1], is located at the northern tip of the supercluster. Further, rich clusters are situated in the western and southern tips—the Pegasus cluster U487[8] and A194, respectively. Other clusters form chains joining the tips of the supercluster. A chain is located close to the Milky Way, forming the northern boundary of the Andromeda supercluster.

In Fig. 1*b* two superclusters can be seen: the Pegasus supercluster in the right-hand section of the figure and the Cetus supercluster in the lower left-hand section. In the central area of Fig. 1*b* there are no clusters. This 'big hole' is surrounded by superclusters: the Perseus supercluster forms a wall at the near side of the hole, superclusters seen in Fig. 1*b* form walls at the left and right sides, and superclusters at redshifts $10{,}000 \le V_0 < 15{,}000$ km s^{-1} form walls at its far side.

The distribution of redshifts of galaxies in the central area of the Perseus supercluster (where there are no clusters) is given in Fig. 2*a*; Fig. 2*b* shows the distribution of redshifts of clusters of galaxies in the whole area of the Perseus supercluster, and Fig. 2*c* shows the distribution of redshifts of clusters in the remaining area outside the Perseus supercluster. There is a very strong peak in Fig. 2*a* and *b* at the redshift 5,000 km s^{-1} due to the Perseus supercluster. The distribution of redshifts at large redshift values is similar for galaxies and clusters. This suggests that galaxies situated in the central area of the Perseus supercluster form a thin stratum at the same distance as clusters form chains. The distribution of redshifts in Fig. 2*c* is completely different since it is caused by other superclusters situated at a greater distance from us.

In the direction of the Perseus supercluster there are only a few foreground and background galaxies, the vast majority of galaxies of magnitude $13.0 < \text{mag} \le 15.0$ belong to the Perseus supercluster. Thus we can use galaxy counts to estimate some numerical properties of the Perseus supercluster. The results are presented in Table 1. *N* denotes the number of Palomar Observatory Sky Survey fields, *L* the luminosity calculated by the method suggested by Jôeveer, Einasto and Tago[3], *B* the surface brightness, and %S is the per cent of spiral galaxies in the respective area. Morphological types of galaxies were taken from Nilson[8]. In the right-hand column the number of radio galaxies is given[9].

The data presented here indicate that the Perseus supercluster of galaxies is surrounded by chains of clusters of galaxies which join main density peaks—rich clusters. Main peaks have a low percentage of spiral galaxies (most galaxies are elliptical, or S0 galaxies) and contain many radio galaxies. In the central area of the supercluster the density of galaxies is small, there are no clusters here, and almost all the bright galaxies are spirals.

The total area covered by the Perseus supercluster is 5,400 Mpc^2, its mean thickness is 7 Mpc, volume 3.8×10^4 Mpc^3

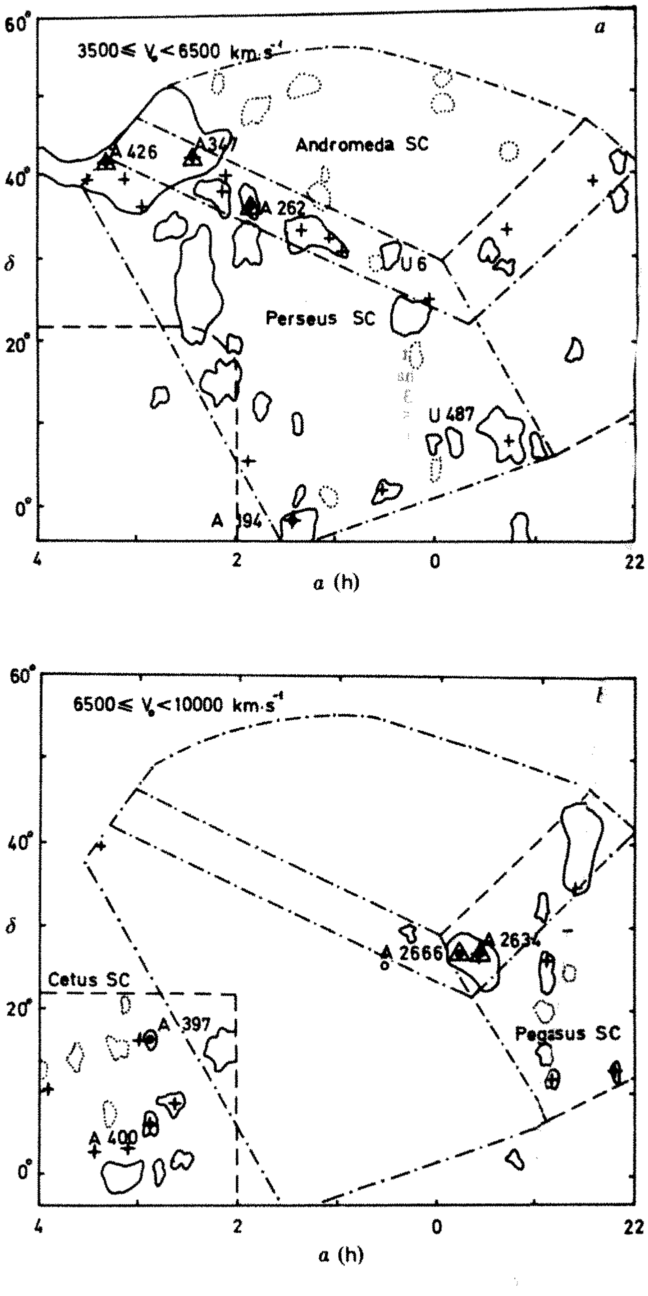

Fig. 1 The distribution of Zwicky clusters of redshifts $3{,}500 \le V_0 < 6{,}500$ km s^{-1} (*a*), and $6{,}500 \le V_0 < 10{,}000$ km s^{-1} (*b*). Zwicky clusters with measured redshifts have been drawn by solid lines, the clusters with estimated redshifts by dotted lines. Abell clusters are shown as filled circles, radio galaxies as crosses, and X-ray sources as triangles. Approximate boundaries of superclusters are shown as dashed and dotted-dashed lines.

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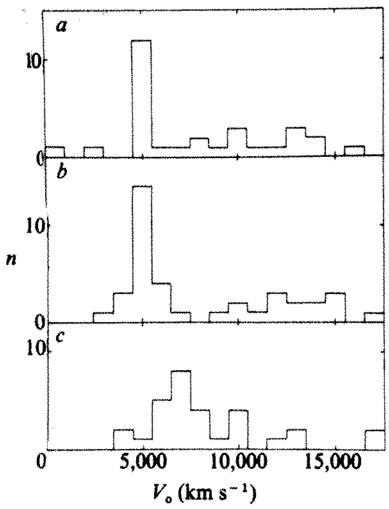

Fig. 2 The distribution of redshifts of galaxies in the central area of the Perseus supercluster (10 Palomar survey fields) (*a*), Zwicky clusters in the whole Perseus supercluster area (*b*), and Zwicky clusters outside the Perseus supercluster area (*c*).

and mass (adopted mean mass-to-luminosity ratio 150) $2.7\times 10^{16} M_\odot$. The mean density in the supercluster is 10 times greater than the critical cosmological density[6].

Neighbouring superclusters are in contact and contain common elements. For example, the chain of clusters between clusters A426 and U6 forms the northern boundary of the Perseus supercluster and the southern boundary of the Andromeda supercluster.

In the area under study in redshift interval $3{,}500 \leq V_0 < 10{,}000$ km s^{-1} essentially all clusters are members of four superclusters (see Fig. 1). The space between superclusters (about 98% of the total volume studied[6] contains no clusters and very few bright galaxies.

A nonlinear theory of the growth of inhomogeneities suggests that the amount of matter concentrated in superclusters (initial positive density perturbations) is only a little larger than the total mass of the dark matter in big holes (initial negative density perturbations)[6]. If the density of matter associated with superclusters is $\Omega_{sc} = 0.2$ (ref. 3), then the overall mean matter density is $\Omega = 0.3$. The different large-scale spatial distribution of dark and luminous matter cannot be reconciled with pure gravitational clustering theories[10].

On the other hand, the morphology and structure of superclusters suggest that it is impossible to form superclusters in a wholly gaseous medium. In a gaseous proto-supercluster, the formation of galaxies has to begin in the central part of it, and would lead to a large cluster of galaxies rich in ellipticals in the centre of the supercluster[11]. Towards the edges the supercluster would be much thinner, and most galaxies in this region would be spirals. The observed structure is just the opposite.

Table 1 Data on the Perseus supercluster

Supercluster region	N	L ($10^{12}L_\odot$)	B ($10^9 L_\odot$ Mpc^{-2})	%S	No. of radio galaxies
Mean peaks	3	30	79	52	4
Cloud around A426	8	39	39	58	5
Perseus chain	7	60	68	68	6
Other chains	15	43	23	79	2
Central area	10	9	8	≥95	0
Total	43	181	33	68	17

This difficulty can be avoided if, following Rees[12,13], we suppose that the formation of galaxies was a two-stage process, but also that it involves much larger spatial dimensions than Rees suggested. In the first stage proto-superclusters and big holes had to form from the non-dissipative dark matter[14]. As demonstrated by numerical simulations non-dissipative matter would eventually form the observed structure with high density tips, intermediate density chains and low density walls, as long as the initial spectrum of perturbations contains large scale components[15]. In the second stage, hot gas, by cooling and settling down into the potential wells caused by dark matter, will form galaxies and clusters of galaxies[16].

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The source of the Jupiter S-bursts

G. R. A. Ellis

Department of Physics, Box 252C, GPO, Hobart,
Tasmania, Australia 7001

The transient radio S-bursts from Jupiter are strongly correlated with the orbital position of the satellite Io for all wave frequencies between 8 MHz and 30 MHz, and with system III longitude above about 15 MHz. The observations are most easily explained if the electrons generating the S-bursts are confined to the planetary magnetic flux tube intersecting Io (the IFT)[1]. The primary source of the electron energy is thought to be the electromotive force (EMF) generated by the relative motion of Io across the Jupiter magnetic field, the S-bursts being the result of convective instabilities in the consequent current flow in the IFT. The electrons are assumed to emit coherent electromagnetic radiation in the Doppler cyclotron mode as they travel up the IFT after being accelerated near its feet in the Jupiter ionosphere to energies of a few keV (refs 2–4).

Simple calculations using the observed rate of change of wave frequency with time for the S-bursts ($df/dt \sim -10$ to -20 MHz s^{-1}) give 3–12 keV for the electron energy, and observations over a long period have always yielded electron energies in this range[5]. The narrow bandwidth of individual bursts ($\Delta f \sim 10$ kHz)[6] implies that their velocity spread is very small ($\Delta v/v \sim 10^{-3}$), so that the acceleration mechanism must be fairly stable and almost monoenergetic. At the same time, the magnetic field intensity in the IFT will vary as Jupiter rotates under Io owing to the non-centred location of the Jupiter

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magnetic dipole[7]. Some variation in the dynamical properties of the electrons correlated with the longitude of Io, and consequently in the properties of the S-bursts, might hence be expected.

The S-bursts were observed between 1972 and 1979 in the frequency range 2.5–17 MHz with the 650×650 m Llanherne telescope, in the frequency 17–29 MHz with a 60×60 m array, and at 24–35 MHz using a new 85×170 m array of 4,096 dipoles. RF signals were recorded on video tape for analysis with a 256-channel spectrum analyser with frequency resolution 10 kHz and time resolution 600 μs. Examples of S-burst spectra at 9, 16, 22 and 32 MHz are shown in Fig. 1. The observed distribution of df/dt with Io longitude for all the bursts observed near 12 and 15 MHz is shown in Fig. 2. Each point gives the mean value and standard deviation of df/dt during each 5-min daily observation period. Although df/dt varied between -6 and $-15\,\mathrm{MHz\,s^{-1}}$ during the period of the observations, the variation on any particular occasion was much less, with the ratio of standard deviation to the mean being typically 0.1. That is, all the bursts, of the order of a thousand, observed in 5 min on a particular day had similar frequency–time slopes but varied considerably from day to day. In Fig. 3, the observations of df/dt at various wave frequencies between 5.5 and 35 MHz are shown alternatively as a function of frequency for two regions of system III (1965) sub-Io longitude L with $180^\circ < L < 260^\circ$ and $320^\circ < L < 040^\circ$, chosen for high and low values of the magnetic field B_0 at the foot of the IFT.

If it is assumed that for emission at a particular wave frequency, taken to be the cyclotron frequency, the electrons are

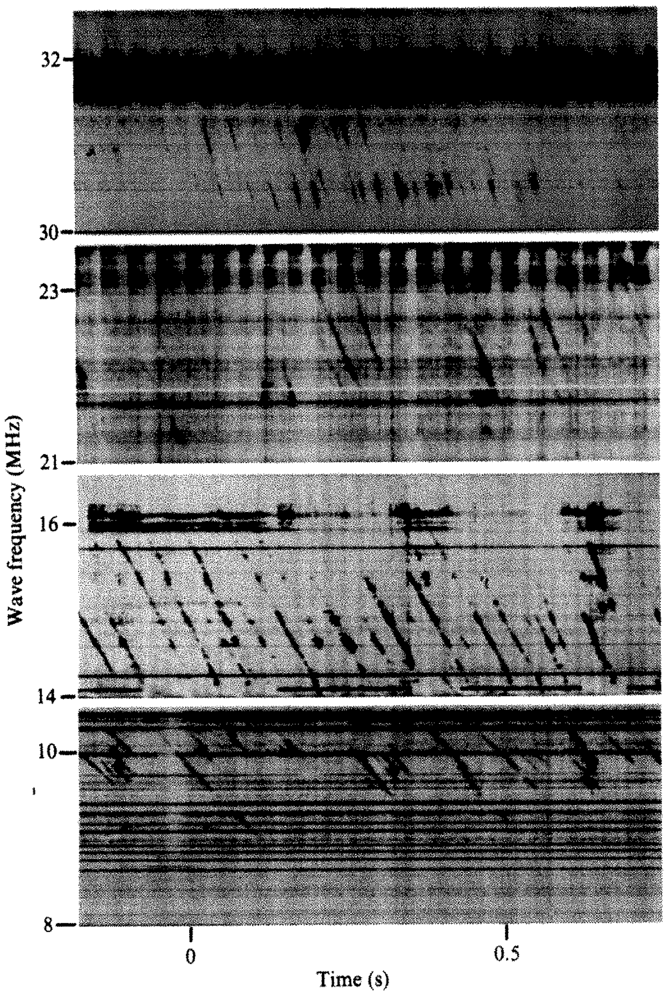

Fig. 1 Dynamic spectra of S-bursts near wave frequencies of 9, 15, 22 and 32 MHz showing their increase in frequency–time slope with frequency.

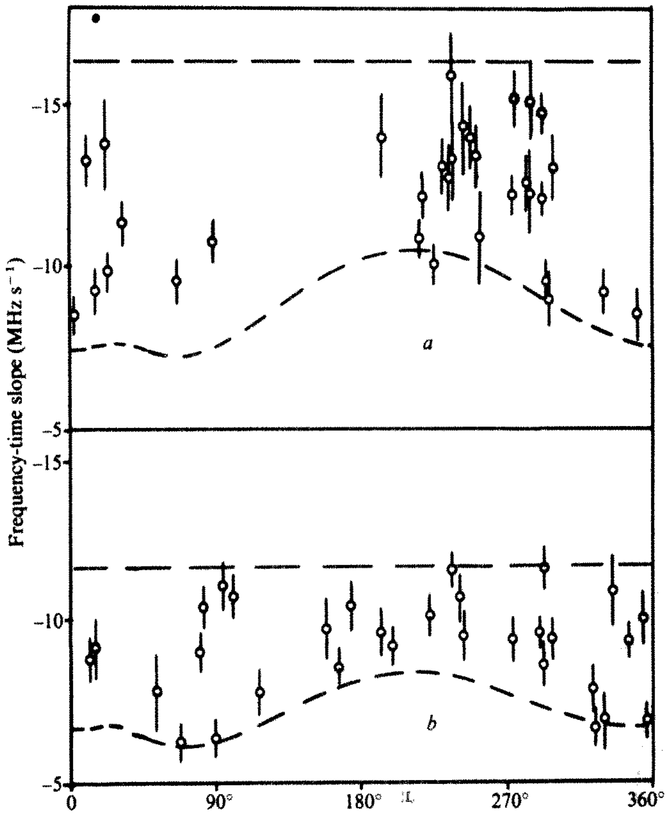

Fig. 2 Observed variation of the df/dt of S-bursts with system III longitude of Io. *a*, Wave frequency 15 MHz. *b*, Wave frequency 12 MHz. In each section the upper and lower dashed lines show the limits expected for df/dt with electron energy 3 keV and initial pitch angles 0 and $\pi/2$ respectively.

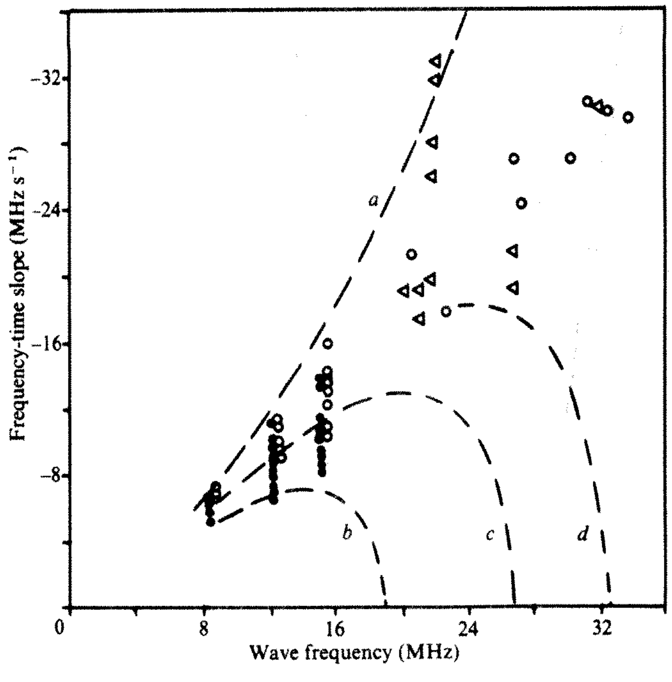

Fig. 3 Observed variation of df/dt with wave frequency from 8.5 MHz to 34 MHz. ●, Present observations, $320^\circ < L < 040^\circ$; ◁, observations reported by Desch, Flagg and May[3], $180^\circ < L < 260^\circ$; ○, present observations $180^\circ < L < 260^\circ$. The dashed lines (*a*) and (*b*) show the calculated limits of df/dt for $320^\circ < L < 040^\circ$, and (*a*) and (*c*) for $180^\circ < L < 260^\circ$. The line (*d*) shows the lower limit of df/dt expected for northern hemisphere sources only ($180^\circ < L < 260^\circ$). Electron energy, 3 keV.

all accelerated in the ionosphere to the same energy, then their longitudinal velocity at the point of S-burst emission will depend only on their initial pitch angle ϕ_0 and on B_0. That is,

$$\frac{\mathrm{d}f}{\mathrm{d}t} = -KV_{\parallel} = -KV\left(1-\frac{B}{B_0}\sin^2\phi_0\right)^{1/2}$$

where K is constant and B is magnetic field at emission point. The maximum and minimum values of $\mathrm{d}f/\mathrm{d}t$, obtained by taking $\phi_0 = 0$ and $\pi/2$, will vary with wave frequency $f(\propto B)$ and with B_0 and hence Io longitude. Figure 2 shows how the limits of $\mathrm{d}f/\mathrm{d}t$ calculated in this way vary with longitude for wave frequencies 12 MHz and 15 MHz. In Fig. 3, the same limits are shown as a function of wave frequency for the two restricted longitude regions specified earlier. Both northern and southern hemisphere values of B_0 were used for wave frequencies below 17 MHz since polarisation observations have shown that in this frequency range S-bursts may have either right-hand or left-hand polarisation[9] and may presumably originate in either hemisphere. Above 17 MHz, northern hemisphere sources were assumed.

It can be seen that all the observed values of $\mathrm{d}f/\mathrm{d}t$ fit reasonably well between limits calculated using an electron energy of 3 keV whether they are plotted against Io longitude or wave frequency. That is, with this model only a single value of electron energy with a range of initial pitch angles from 0 to $\pi/2$ is needed to account for the observed wide variation of $\mathrm{d}f/\mathrm{d}t$. The observations suggest that the electron acceleration is stable over long periods, a result which might be expected from the properties of the Io unipolar inductor[4].

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Changes in the optical light curve of the Crab pulsar between 1970 and 1977

D. H. P. Jones*, F. G. Smith* & J. E. Nelson†

* Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, East Sussex, UK
† Lawrence Berkeley Laboratory, Berkeley, California 94720

The pulsed optical radiation from the Crab pulsar has a characteristic and well determined profile. As the pulsar is comparatively young, the slope of this profile may be expected to change appreciably within only a few years. Our observations over an interval of 7 yr show that any such changes are surprisingly small, amounting to <1% of the peak pulse intensity.

The two profiles of the pulsed optical radiation from the Crab pulsar were obtained as follows, in sufficiently similar circumstances to allow us to search for small differences. The first observations by Nelson *et al.*[1] were made at the Lick Observatory between 15 May 1969 and 3 May 1970 using the 0.9-m Crossley reflector. Figure 1 shows a composite of the data which were collected in 25-μs intervals but shown here in 250-μs intervals—in groups of 10 bins. The data cover 72% (23.8 ms) of the cycle. The second observation was made by Smith *et al.*[2] on 26 January 1977 with the 3.9-m Anglo-Australian telescope, using an FW 130 photomultiplier with S20 response. These latter data were collected in 20-μs intervals.

The spectral responses of the two sets of observations were similar, and although no special precautions were taken to avoid sensitivity to polarisation effects, we do not consider that these could seriously affect our results. Because of the similarity of the pulse profile at different wavelengths[3] any difference in spectral response would have to be enormous to affect the results.

After allowing for the lengthening of the period by 1 part in 350, the light curves are remarkably similar. Bearing in mind that the intensity zeros and the relative phases of the two curves are unknown, we decided to fit the main peaks as closely as possible and look for any remaining differences. We first adopted a time in the Smith data corresponding to the first interval in the Nelson data. For each Nelson bin the corresponding point in the time base of the Smith curve was found by multiplying the bin number by

$$\frac{25}{20}\times\frac{0.033\,200\,3858}{0.033\,106\,1538}$$

that is, by the product of the ratio of bin sizes and the ratio of the periods[4]. The Smith count corresponding to each Nelson bin was then found by four-point lagrangian interpolation between the four Smith bins closest to the Nelson bin. It can easily be shown that, in the mean, this interpolation reduces the effective dispersion in the Smith observations by a factor 0.77. The Smith curve was then regressed on to the Nelson curve over ±6 ms centred on the main pulse. The whole procedure was repeated for a series of time-offsets between the two light curves in search of the minimum r.m.s. fit. The differences between the two complete light curves after the best fit had been obtained on the main peak are shown in Fig. 2. The data in Figs 1 and 2 are plotted in bins 250 μs wide, as we find that the differences are too small to show clearly in 25-μs bins. The scatter of points in Fig. 2 is somewhat wider than would be expected from statistics alone.

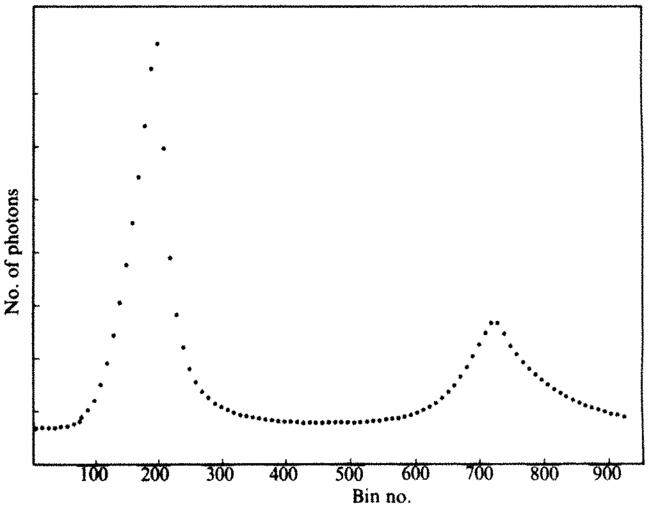

Fig. 1 Nelson *et al.*[1] light curves of the Crab Nebula pulsar. The original 25-μs bins have been averaged in groups of 10.

The two main pulse profiles evidently have a very similar shape. There are, however, two outstanding points on the steepest part of the falling slope 0.4 ms after the peak (bins 211–220 and bins 221–230). Both these show a 1% discrepancy from the intensity at that part of the curve, which is well above the statistical uncertainty. Figure 3 shows the data from the individual 25-μs bins in this region: the smoothness of the curve indicates that the difference may be real, representing a slower rate of fall in the later light curve.

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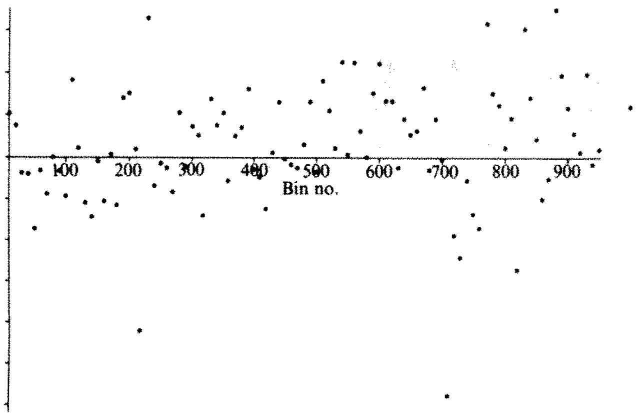

Fig. 2 The difference between the Smith *et al.*[2] curve and the 1970 curve. The ordinates have been averaged over 10 bins.

Apart from this small effect the shape of the main pulse has not changed significantly; the other differences between bins 0–300 are scattered fairly randomly about zero. No significant improvement in the two discrepant bins can be made without seriously worsening the fit elsewhere in the main pulse.

Using this best fit, three other differences can be seen in the rest of the light curve as follows: (1) base level changes: the low levels before the main pulse and the interpulse cannot both be fitted by our procedure. As the intensity is least before the main pulse[2,5], we interpret the change in terms of a constant level at this point; the result is then an increase in the level between the main pulse and the interpulse. The increase is 0.15±0.05 (s.e.)% of the peak intensity above minimum, appearing between bins 500 and 700.

(2) Relative intensity of pulses: the low values between bins 710 and 760 represent a decrease of interpulse intensity of 0.91±0.25 (s.e.)%.

(3) Spacing of pulses: the low values are not uniformly distributed around the peak of the interpulse. The interpretation of this is uncertain, as there is an irregular spread of comparatively large difference values in the region of the interpulse. A better fit for the interpulse is obtained if the spacing between main pulse and interpulse is increased by 15 μs, but it is doubtful whether this is significant. Any relative movement of the pulses cannot be greater than ~25 μs, corresponding to about 0.25° of pulsar rotation phase.

An interval of 7 yr in the life of the Crab pulsar, which is <1,000 yr old, might be expected to produce appreciable changes in the light curve. We find that the only significant changes amount to <1% of the peak intensity: they appear as a small change in the steep fall-off from the main peak, a change in the relative levels of the baseline before and after the main peak, and a decrease in the interpulse intensity relative to the main peak.

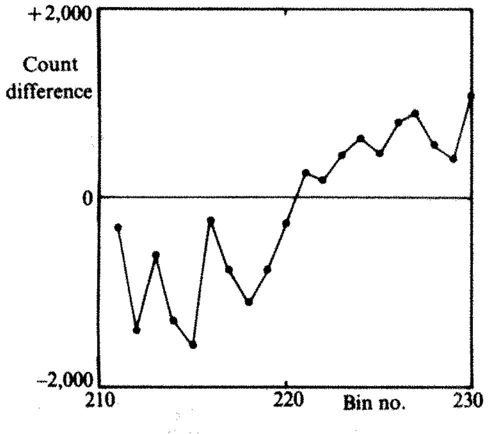

Fig. 3 Differences for 25-μs bin numbers 210–230, showing a change in the falling portion of the main pulse.

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The frictional anisotropy of diamond

Y. Enomoto* & D. Tabor

Physics and Chemistry of Solids, Cavendish Laboratory, Madingley Road, Cambridge, UK

If a diamond stylus is slid over a flat diamond surface in air the friction may depend significantly on the direction of sliding. The most marked effect is on the {100} faces. Along the ⟨100⟩ direction the friction is high (coefficient of friction $\mu \simeq 0.15$) while along the ⟨110⟩ direction it is low ($\mu \simeq 0.07$). This has been variously attributed to surface roughness (Coulomb friction) or to processes involving surface adhesion. (Some of these processes and related ideas are discussed in ref. 1.) We describe here some recent experiments which show that frictional anisotropy occurs only when the contact pressure exceeds a critical value. In these conditions cathodoluminescence studies of the diamond flat indicate that the frictional anisotropy is largely due to surface and sub-surface damage produced in preferred crystallographic directions by the sliding process itself. However, the results do not, as yet, provide a detailed account of the mechanism of energy dissipation.

The experiments were carried out with a very simple apparatus and involved the sliding of diamond styli over the polished (001) surface of Type I and Type II diamond. The flat surface was cleaned in air with solvents so that it was covered with oxygen and possibly other adsorbed contaminants. The styli were hemispherical sliders, of radius of curvature 18, 80 and 140 μm. The normal load could be varied from 4.9 mN (0.5 g) to 19.6 N (2,000 g) and the sliding speed was 0.2 mm s^{-1}.

The Type I diamond was polished to within 1° of a (001) plane. Polishing lines, parallel to the ⟨100⟩ direction, were visible in the optical microscope and about 1 μm apart. The Type II diamond was several degrees off the (001) plane and the polishing lines were somewhat finer. Both diamonds showed characteristic frictional anisotropy the lowest friction being along the ⟨110⟩ directions and the highest along the ⟨100⟩ directions. With the Type I diamond the frictional anisotropy was not quite symmetrical, the friction in the [010] direction being higher than in the [100]. The reasons for this will be discussed in a later, more detailed publication. With Type II diamond the frictional anisotropy was symmetrical as shown in Fig. 1. It is seen that at a load of 0.2 N (20 g) the coefficient of friction in the ⟨100⟩ directions is about 0.12 and about 0.07 in the ⟨100⟩ directions. This is not a Coulomb type of behaviour where the friction is attributed to the climbing of one surface over asperities on the other, for in such a situation the coefficient of friction is determined solely by the surface topography and is independent of the load. In contrast, in the present experiments, if the load is reduced to 0.05 N (5 g) the anisotropy disappears. This suggests that the frictional anisotropy is associated with damage produced in the flat diamond surface when the contact pressure exceeds some critical value discussed below.

This was confirmed by experiments carried out on the polished (001) surface of a Type II diamond using a stylus of radius $R = 140$ μm and loads ranging from about 4.9 mN (0.5 g)

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* Present address: Mechanical Engineering Laboratory, 1-2 Namiki, Sakura-mura, Niihari-gun, Ibaragi-ken, Japan.

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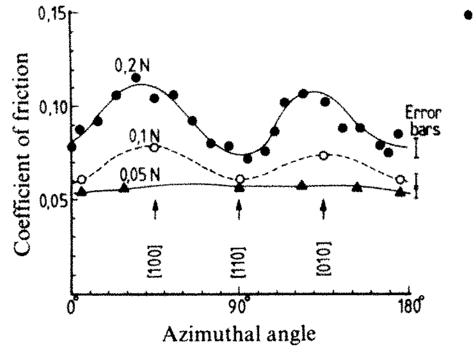

Fig. 1 Coefficient of friction μ of a diamond stylus (radius of curvature 18 μm) sliding over the (001) face of a Type II diamond. At a load of 0.2 N (20 g) the friction along the [100] direction is nearly twice the friction along the [110] direction. The frictional anisotropy is not due to a Coulomb type of friction since if the load is reduced to 0.05 N (5 g) the anisotropy disappears. Similar effects were also observed on the Type I diamond.

to 19.6 N (2,000 g). At low loads the friction ($\mu \simeq 0.07$ to 0.08) was essentially the same in the [100] and [110] directions and did not increase appreciably with load. However, in the [100] direction there was a sudden rise in friction when the load exceeded a critical value W_c: for example, at a load of 11.8 N (1,200 g) μ reached a value of 0.14. The tracks formed in this direction of sliding were examined by cathodoluminescence which reveals surface and subsurface damage. Figure 2 shows that as the damage increases, with increasing load, the friction increases. (Note that a load $W = 6.9$ N where there is a slight diminution in the amount of damage the friction shows a small drop).

A study was also made of the critical load W_c at which a marked rise in friction in the [100] direction occurred as a function of the radius of curvature R of the slider. Our experiments showed that W_c was closely proportional to R^2. If we assumed that this transition is associated with the generation of appreciable subsurface damage we may apply the principles of fracture mechanics. Frank and Lawn's[2] work on the growth of cracks in brittle solids shows that for static contact W_c should be proportional to R (Auerbach's law). On the other hand Lawn[3] has shown that in sliding contact for $\mu > 0.02$ W_c should be proportional to R^2. Our results therefore qualitatively agree with Lawn's theory and quantitatively agree if it is assumed that there are initial cracks in the surface of the order of 0.5 μm deep. The proportionality between W_c and R^2 implies that for elastic (hertzian) contact, the friction transition occurs when the mean contact pressure reaches a critical value: in our experiments this has a value of order 2,000 kg mm^{-2}.

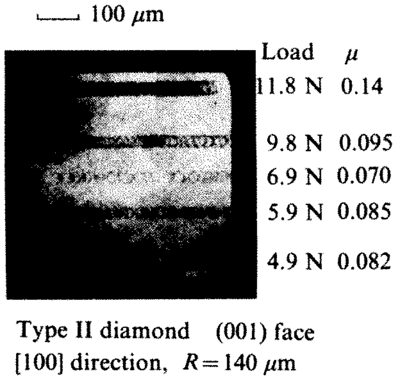

Fig. 2 Cathodoluminescence observed on the (001) face of a Type II diamond after a single to-and-fro traversal with a diamond stylus of radius $R = 140$ μm. The corresponding loads and coefficients of friction μ are indicated. There is a marked increase in subsurface damage and in μ at a load of 11.8 N (1,200 g). Scale bar, 100 μm.

We conclude that the frictional anisotropy is, to a large extent, due to surface and subsurface damage produced in preferred crystallographic directions by the sliding process itself. It is possible that some plastic grooving occurs in the friction track though the pressure of 2,000 kg mm^{-2} seems too small for this. On the other hand some microplastic deformation may be involved and contribute to the energy dissipation but the major factor apparently involves subsurface cracking which is generated when the contact pressure exceeds some critical value. This is consistent with our observations that with softer styli such as steel or even tungsten carbide frictional anisotropy is not observed over an extremely wide load range. It is also consistent with the practical observation that in the polishing of diamond with diamond powder the polishing rate is very much higher in the directions of high friction presumably because of the increased ease of producing cracks in and beneath the surface. Although these results explain most of the relevant phenomena they do not yet provide a detailed account of the way in which the energy is dissipated.

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Observations on internal structures of chrome alum dehydration nuclei

A. K. Galwey & R. Reed

Queen's University of Belfast, Belfast, UK

G. G. T. Guarini

Istituto di Chimica Fisica, Università di Firenze, via G. Capponi, 9, 50121, Firenze, Italy

Decomposition reactions of many solids proceed by nucleation and growth processes[1], so that the kinetic expressions used[2] and the interpretation of rate data[3] are based on quantitative consideration of the progressive changes in geometry of advancing reactant/product interfaces. Conclusions from such kinetic analyses are often confirmed by optical microscopic observations. We describe here a method whereby information may be obtained, in favourable systems, about the three-dimensional shapes of nuclei and the disposition of product particles in the wake of interface advance. For the reaction described below, the dehydration of $KCr(SO_4)_2 \cdot 12H_2O$, it is concluded that product shrinkage results in crack development through which water is evolved and crystallographic factors exert little control on the directions of interface advance during growth of the approximately hemispherical nuclei.

Most previous microscopic studies of solid state decompositions have been restricted to measurements of the numbers, distributions, sizes and shapes of nuclei within the two-dimensional plane that constitutes the crystal boundary surface. Such information is often an incomplete representation of the nuclei which may be three-dimensional assemblages of large numbers of small product particles. A new technique is described here whereby, in suitable systems, information can be obtained on

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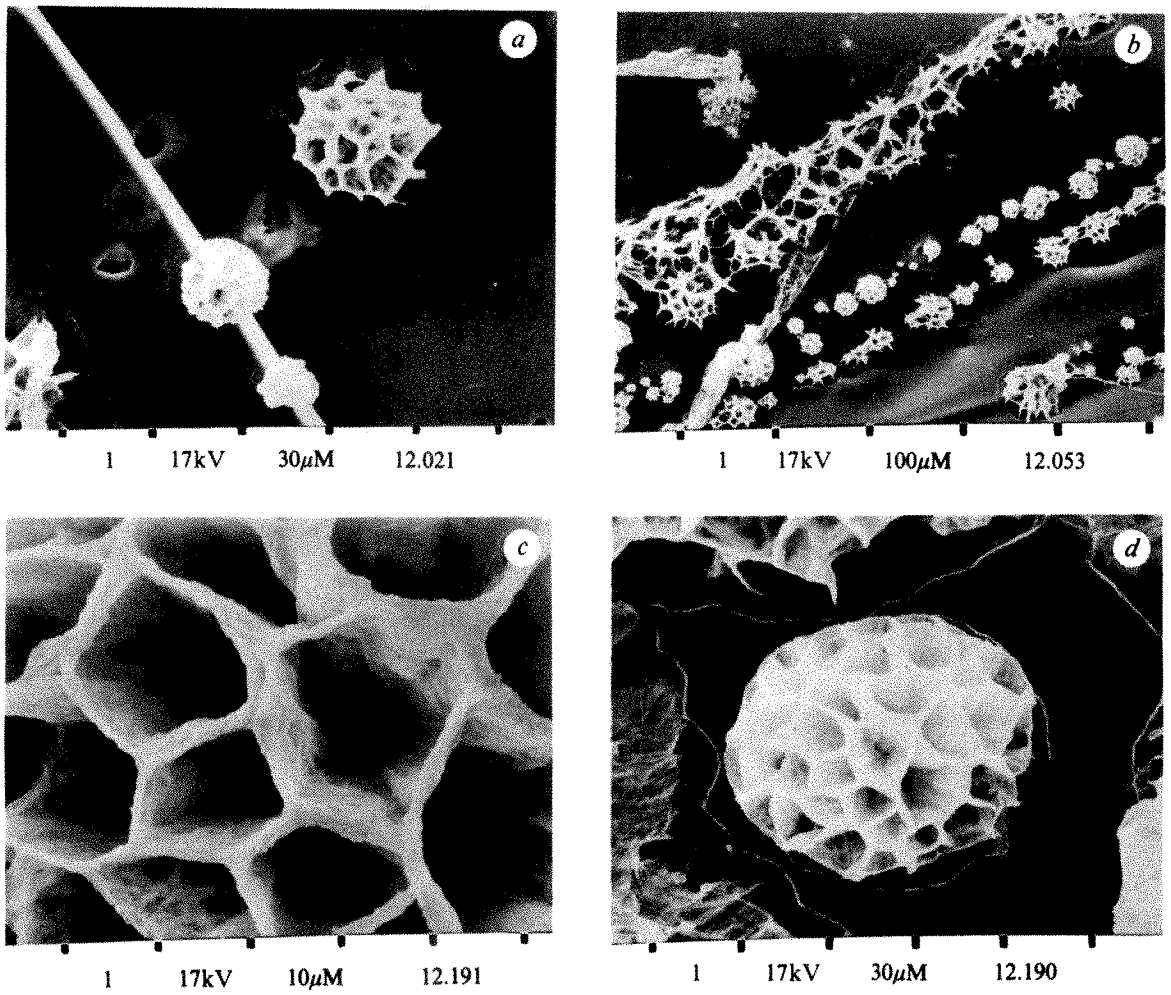

Fig. 1 Scanning electron micrographs of replicas of nuclei developed on cleavage surfaces of chrome alum, partially dehydrated in vacuum, prepared by techniques described in the text. Magnification as shown by scale on photographs. *c* Is an area of *d* more highly magnified.

the texture of the product phase, including particle sizes and any topotactic relationship with the reactant. Such evidence is particularly valuable in the formulation of mechanisms of bond and lattice rearrangement steps within the restricted zone that is regarded as constituting the active reaction interface. A knowledge of crack dimensions may permit estimation of the impedance offered to volatile product escape during reversible reactions. Present mechanisms of interface processes are usually inferred from indirect evidence such as Arrhenius parameters[1].

Figure 1 gives evidence of a regular internal crack development in the characteristic nuclei evolved during the vacuum dehydration of chrome alum ($KCr(SO_4)_2 \cdot 12H_2O \rightarrow KCr(SO_4)_2 \cdot 6H_2O + 6H_2O$; this reaction was previously studied by Garner *et al.*[4]). These are scanning electron micrographs of replicas of the nuclei, which are apparently 'seen' from a viewpoint inside the crystal. The solid replication material fills the voids within the product assemblage that constitutes the nucleus. These photographs were obtained as follows. Large (~5 mm edge) solution-grown chrome alum crystals were cleaved by a sharp blow with a knife edge and acceptable planar surfaces were dehydrated 10–30 min in vacuum ($\sim 10^{-4}$ torr) at ~300 K. The development of nuclei was confirmed by optical microscopic examination and, with minimum exposure to the atmosphere, the crystal was withdrawn from the vacuum and a drop of 2% solution of polyvinyl formal solution in dry chloroform carefully placed only on the surface of interest. After solvent evaporation and hardening of the replica (>24 h), the alum crystal was removed by dissolution in distilled water, the replica washed, dried and coated with gold–palladium before examination in the Stereoscan S180. (Alum crystals are not sufficiently stable to be directly examined in the microscope.)

Figure 1 shows representative features of typical nuclei on surfaces present after partial dehydration of cleavage surfaces of chrome alum. Figure 1*a* includes a typical nucleus having a divergent distribution of laminar cracks between product assemblages of approximately hexagonal section. At the crystal boundary plane it is possible to identify the approximately circular outline of the nucleus, locally curving outwards in the vicinity of advancing cracks, that is seen by optical examination of the crystal surface[1,4]. The oblique line is a surface step or crack, at which two nuclei have been preferentially generated, though along much of this zone and at other sites of imperfection reaction has not, as yet, been initiated. Figure 1*b* shows that there are strongly preferred directions of alignment of nuclei. Nuclei in the upper aligned band have overlapped with interlinking of volatile product escape channels. The crack systems in the lower two parallel lines of nuclei have started interpenetration but overlap is, as yet, less extensive. The crack systems characteristic of a developed individual nucleus are shown at two magnifications in Fig. 1*c* and *d*.

The development of strain during dehydration[5] is believed to result in the generation of this internal regular hexagonal disposition of cracks within nuclei, which is somewhat similar to that found in columnar basalt[6] where it has also been ascribed to shrinkage. Crystallographic factors are of little significance in controlling the direction of interface advance during growth of hemispherical nuclei in this alum.

We suggest that examination of the internal structures of nuclei, as revealed by this replication technique, provides a new approach to the elucidation of the mechanisms of solid state reactions. Evidence from the structures of dehydration nuclei developed in $KCr(SO_4)_2 \cdot 12H_2O$ supports earlier suggestions[5] that stress is an important factor in controlling product formation: a more detailed consideration of this and related reactions will be published elsewhere.

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Palaeointensity and thermoluminescence measurements on Cretan kilns from 1300 to 2000 BC

Yannis Liritzis & Ruth Thomas

Departments of Physics and Geophysics, University of Edinburgh, Mayfield Road, Edinburgh, UK

The strength of the geomagnetic field in Crete during Minoan times has been determined using kiln wall samples from three Minoan palaces, Faistos, Aghia Triada and Kato Zakros, from the Minoan village at Stylos Hania and a Roman kiln at Kalo Horio. The samples have been dated by thermoluminescence measurements providing absolute dates for the construction of a palaeointensity curve. Without the thermoluminescence dates it would have been necessary to use the archaeologists' estimates of the kiln ages, one of which is shown here to be in error by 500 yr.

Kiln material was used because: (1) pottery was not readily available; (2) it was hoped that both palaeointensity and palaeodirection results could be obtained; and (3) the last firing events which were related to the destruction of the Minoan palaces could be dated by thermoluminescence measurements.

The samples, from the kiln floor, walls and heat channels, consist of a fired calcareous mud, varying in appearance from the extremes of a well fired vesicular slag (Aghia Triada and site (1) Faistos) to orange, porous, friable, less well-fired, samples with a fine mud matrix containing fragments of shells, rocks and pottery (site 1 of Kato Zakros, Stylos Hania and site 3 of Faistos); the remaining samples (Kalo Horio and site 1 of Faistos) contain a mixture of both types. Kato Zakros (2) is a brick fragment and Kato Zakros (3), a tile fragment.

The palaces sampled were of middle and late Minoan age. The earliest palace site sampled was Faistos. Kiln FS (3) belongs to the first palatial period (~2000–1700 BC) which ended when the palace was destroyed by earthquakes in about 1730 BC (ref. 1). The palace was subsequently rebuilt but in about 1500 BC it was destroyed by earthquakes or, according to some authors, by the effects of a volcanic eruption of Santorini (refs 2–5). Kiln FS (1) belongs to this second palatial period. The palace of Aghia Triada was built to replace the second palace at Faistos and probably lasted to 1400 BC (ref. 6).

The palace at Kato Zakros is thought to date from 1700 to 1400 BC. Platon (personal communication) believes that it was not rebuilt or inhabited after the catastrophic earthquake which occurred between 1450 and 1400 BC. The kiln at Stylos Hania is believed to be of late Minoan age[7] (~1300 BC); no pottery was found in the kiln but the nearby village is of that age. Kalo Horio, a Roman kiln was believed to date from ~50 BC.

Table 1 Thermoluminescence ages (yr BP)

Site	Age of sample	Estimated % error	Average site age	Estimated % error
Stylos Hania	3,882	6	3,828	4
	3,840	6		
	3,763	7		
Faistos (1)	3,466	6	3,466	6
Faistos (3)	3,806	5	3,784	4
	3,762	6		
Aghia Triada	3,545	6	3,508	4
	3,472	5		
Kato Zakros	3,370*	5	3,303	2
	3,199*	5		
	3,139*	6		
	3,289	5		
	3,519	5		
Kalo Horio	2,126	7	1,960	5
	1,795	7		

* These samples are tiles, all other samples are from kilns.

The thermoluminescence (TL) method used was the quartz inclusion technique described by Fleming[8]. TL dates can only be produced from well-fired materials so the number of dates produced was limited because of vitrification or weathering of the samples. Sample preparation and the determination of the total archaeological dose is described elsewhere[9]. The crystal sensitivity change due to heating to 500°C varied between 5 and 40%. The final total dose error was that derived from the 'dose plateau' tests, the average total doses from two or three peaks only being taken when there was good agreement between them. The β dose rates were measured in two ways, using thermoluminescent dosimetry and also using an α counting and potassium content method. The evaluation of environmental γ radiation was made on site by a portable NaI (T1) scintillometer. Results are presented in Table 1.

One disadvantage of using kiln material in palaeointensity work is that it was not originally selected by a potter and so is more inhomogeneous and complex mineralogically than pottery. Assessment of the mineralogical state of kiln wall material is complicated by the possibility that it has been repeatedly fired and cooled in various gaseous environments.

Attempts were made to determine the palaeointensities by two methods, that described by Shaw[10] and a modified Thellier method[11]. Alteration of one sort or another occurred in all samples used in Shaw's method. For most of the samples < 10% of the NRM remained after demagnetisation at the peak a.c. field of 900 Oe. The ARM2 versus ARM1 gradients were generally <1, except for samples from Aghia Triada, in many cases they had several linear portions of differing gradients.

Table 2 Results using Shaw's method

Site	No. of results	Range of uncorrected palaeointensities (Oe)	Range of corrected palaeointensities (Oe)	Mean and error of palaeointensities (Oe)
Aghia Triada	3	0.369–0.497	0.560–0.590	0.580 ± 0.034
Stylos Hania	5	0.572–0.422	0.365–0.479	0.423 ± 0.035
Faistos (1)	3	0.259–0.786	0.249–0.519	?
Faistos (3)	4	0.776–0.330	0.395–0.333	0.351 ± 0.037
Kato Zakros (1)	5	0.499–0.791	0.474–0.557	0.542 ± 0.058
Kato Zakros (2)	1	ARM and TRM plots nonlinear		
Kato Zakros (3)	3	0.485–0.788	0.512–0.560	0.540 ± 0.048
Kalo Horio	7	0.581–0.887	0.416–0.645	0.505 ± 0.066

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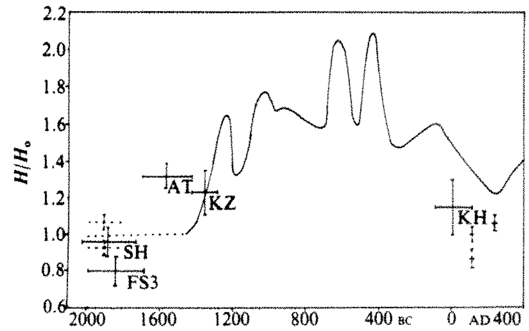

Fig. 1 The change in the ratio of the ancient geomagnetic field intensity H to the present day value, H_0 (0.44 Oe) in Crete. Each point represents several palaeointensity determinations on kiln wall material from one site. The errors are the combined errors from all determinations. The curve produced by Walton[13] is shown for comparison. The Cretan points are labelled, AT, Aghia Triada; KZ, Kato Zakros; SH, Stylos Hania; FS3, Faistos site 3; KH, Kalo Horio. Walton's points which lie away from the smoothed curve are shown by dotted lines.

The TRM versus NRM graphs often showed similar features to the ARM2 versus ARM1 graphs, and where this was the case the correction suggested by Kono[12] was applied to portions of the graph with more than six points. The scatter of results was greatly reduced and for most sites the results were self consistent (Table 2). This method does not always yield reliable results[12] so it was checked using Coe's modified version of Thelliers' method[11]. Alteration of the samples during heating was again a problem and most sites showed a wide scatter of results. The only sites for which useable results were obtained, Kalo Horio (0.506±0.110 Oe) and Kato Zakros 1 (0.484± 0.082 Oe), back up the results obtained using Shaw's method with Kono's correction.

The thermoluminescence results agree with the archaeologists' preferred chronology for the major sites. The result obtained for the less well known site of Stylos Hania suggests that it is older than was previously thought. The thermoluminescence results reinforce the suggested absolute chronology of the Minoan period but cannot rival the detail of relative chronologies deduced by the archaeologists.

The palaeointensity results in Table 2 are compared with those of Walton in Fig. 1. The age error bars on the palaeointensity curve are naturally larger than those of Walton's curve for which the relative ages of the samples are well known. It is unlikely that so precise a curve as Walton's can be extended further back in time, either using archaeological dating or absolute dating which is not yet capable of yielding the necessary precision.

Crete and Athens are ~3° latitude apart; differences in geomagnetic field intensity in the two areas are therefore to be expected. The present value for this difference is 0.01 Oe which is smaller than the errors on the palaeointensity determinations. The maximum difference in geomagnetic field intensity, at present, for sites located at the latitudes of Crete and Athens is 0.03 Oe, so it is to be expected that the palaeointensity results for Crete and Athens will agree within experimental error. This is the case for Stylos Hania and Kato Zakros. The Kalo Horio datum falls at a time where Walton seems to have ignored some data points when constructing his palaeointensity curve. If the Kalo Horio result is to be compatible with Walton data then it is necessary to postulate a fairly rapid change in the geomagnetic field strength.

The palaeointensity results reinforce the thermoluminescence date obtained for Stylos Hania, a palaeointensity of 0.42 Oe being unlikely for a sample aged 1250 BC.

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First measurements of gas phase sulphuric acid in the stratosphere

F. Arnold & R. Fabian

Max-Planck-Institut für Kernphysik, 6900 Heidelberg, FRG

Sulphuric acid is thought to be the most important nucleating agent in the stratosphere and thus has a key role in the formation of the stratospheric aerosol layer ('Junge layer')[1,2]. As the latter at least temporarily influences the Earth's radiation budget and thereby the Earth's climate[3–5], sulphuric acid is a very important stratospheric trace gas. The formation of stratospheric sulphuric acid is considered to proceed via a not yet fully determined photoreaction chain, starting from SO_2 (ref. 6). Sulphur dioxide, in turn, is thought to result from COS and possibly also from CS_2 being transported from the troposphere into the stratosphere[7]. Direct SO_2 injection is also possible. We report here the first measurement of gas phase sulphuric acid abundances in the stratosphere. Our method relies on a recent composition measurement of stratospheric negative ions which revealed the presence of species containing sulphuric acid molecules attached to HSO_4^- core ions[8,9]. Studies of relevant negative ion reactions at the NOAA-Aeronomy Laboratory[10] have confirmed the identifications and reactions of the observed ions, giving a firm basis for determination of stratospheric H_2SO_4 abundances from negative ion composition measurements.

The first composition measurement of stratospheric negative ions showing the major species and their fractional abundances are given in Table 1. The experiment was performed at an altitude of 36.5 km over southwestern France on 10 November 1977. A reaction scheme including the major ions (Fig. 1) suggests that the new cores of the $HSO_4^-(H_2SO_4)_c(HNO_3)_d$ ions are formed by H_2SO_4 reactions (reactions (1) and (4) involving $NO_3^-HNO_3$ and $NO_3^-(HNO_3)_2$. Subsequent H_2SO_4-switching reactions lead to a replacement of HNO_3 ligands by H_2SO_4. While the rate constants of the reactions (1) and (4), have been measured in the laboratory ($k_1 = 8.6 \times 10^{-10}$ cm^3 s^{-1}; $k_4 = 4 \times 10^{-10}$ cm^3 s^{-1})[10], those of the switching-processes have not yet been measured but may safely be assumed to range around 10^{-9} cm^3 s^{-1} with an uncertainty of a factor of ±2. Recombination processes of negative ions with positive ions which are not included in Fig. 1 probably all have rate constants α around 10^{-7} cm^3 s^{-1} at 36.5 km (ref. 11).

Considering the above rate constants the unknown sulphuric acid number density $n(H_2SO_4)$ can be derived from the measured negative ion composition and the measured total positive ion number density n_+ as follows: according to Fig. 1 $NO_3^-(HNO_3)_2$, the most abundant member of the $NO_3^-(HNO_3)_b$ family, is converted via reaction (4) to $HSO_4^-(HNO_3)_2$ which, in

Table 1 Mass numbers, tentative identifications and fractional abundances of the major negative ions observed at 36.5 km altitude

Mass (AMU)	Ion	Abundance (%)
125±2	$NO_3^-HNO_3$	2.6
161±2	$HSO_4^-HNO_3$	5.3
188±2	$NO_3^-(HNO_3)_2$	65.6
197±3	$HSO_4^-H_2SO_4$	6.6
224±3	$HSO_4^-(HNO_3)_2$	14.2
260±3	$HSO_4^-H_2SO_4HNO_3$	3.1
294±3	$HSO_4^-(H_2SO_4)_2$	2.6

turn, is preferably converted to $HSO_4^-H_2SO_4HNO_3$ (reaction(8)) and ultimately to $HSO_4^-(H_2SO_4)_2$ (reaction (12)). Neglecting the thermal decomposition reactions (7), (11) and (13) the steady state continuity equation for the total number density n_S of the product ions $HSO_4^-(HNO_3)_2$, $HSO_4^-H_2SO_4HNO_3$ and $HSO_4^-(H_2SO_4)_2$ becomes

$$(NO_3^-(HNO_3)_2)k_4 n(H_2SO_4) = n_S \alpha n_+ \qquad (1)$$

taking a measured n_+ of 2×10^3 cm^{-3} (ref. 9) which is consistent with a galactic cosmic ray ionisation source and the above α the sulphuric acid number density can be derived from the negative ion abundances (Table 1) using equation (1). The resulting $n(H_2SO_4)$ value is 1.6×10^5 cm^{-3} with an estimated uncertainty of a factor of ±3. Here, typical errors of factors of ±2, ±2, ±1.5 and ±1.5 were considered for k_4, α, n_+ and the negative ion abundance ratio $n_S/n(NO_3^-(HNO_3)_2$. According to Table 1 the neglect of reactions (7), (11) and (13) may have led to an underestimate of $n(H_2SO_4)$ by a factor of 1.4 at the most.

Continuity equations for ions formed from $HSO_4^-(HNO_3)_2$ and $HSO_4^-H_2SO_4HNO_3$ can also be used to derive $n(H_2SO_4)$. However, the rate constants of the switching reactions (8) and (12) have not yet been measured, assuming these to be 10^{-9} cm^3 s^{-1} $n(H_2SO_4)$ values of 8.3×10^4 cm^{-3} and 1.7×10^5 cm^{-3} are obtained having uncertainties which are again a factor of ±3.

If it is assumed that ≈50% of the $HSO_4^-H_2SO_4$ ions were formed via reaction (11) rather than via reaction (5) as suggested by the high observed ratio $n(HSO_4^-H_2SO_4)/n(HSO_4^-HNO_3)$, the former $n(H_2SO_4)$ value of 8.3×10^4 cm^{-3} had to be corrected upwards by a factor of about 1.57 which yields 1.3×10^5 cm^{-3}. Thus the three derived $n(H_2SO_4)$ values are in excellent agreement within the errors quoted. Therefore a sulphuric acid concentration of 1.7×10^5 cm^{-3} with an uncertainty of a factor of ±3 is taken to be representative of our data. The corresponding H_2SO_4 volume mixing ratio is 0.0017 p.p.b.V.

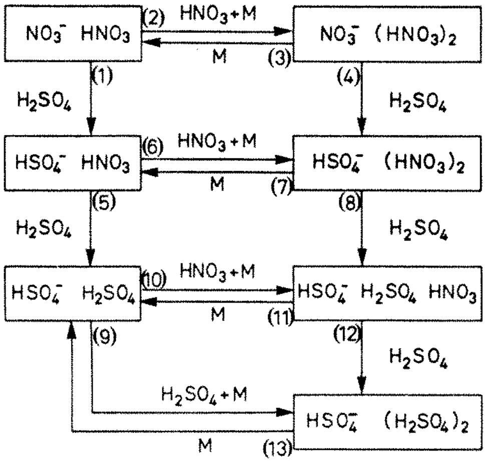

Fig. 1 Reaction scheme including the major negative ions observed. Neutral reaction partners are indicated besides the arrows. M denotes a collision partner.

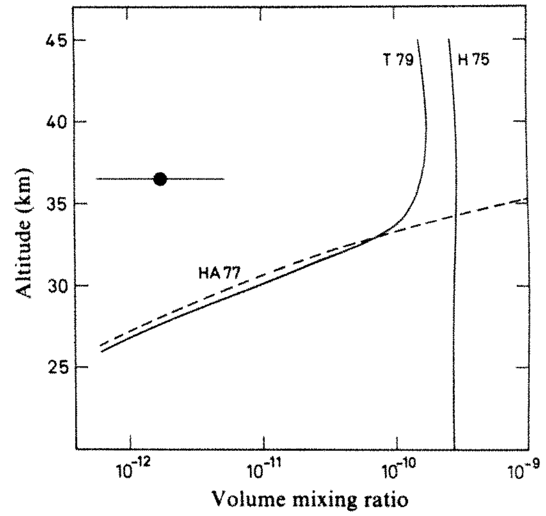

Fig. 2 Volume mixing ratios for gas phase sulphuric acid. ●, The measured value. Profile HA 77 is deduced from vapour pressure data given by Hamill *et al.*[12]. Profile H 75 is given by the photochemical diffusive model of Harker[13]. Profile T 79 is given by the photochemical diffusive model of Turco *et al.*[6] which includes aerosol processes.

This experimental value is compared here with predictions of current models for stratospheric sulphur compounds. Major H_2SO_4 sinks considered are nucleation and photolysis[6]. At heights where the atmospheric temperature is sufficiently low to allow for H_2SO_4 supersaturation, nucleation should be by far the most efficient sink for gas phase H_2SO_4. Then the lifetime of an H_2SO_4 molecule in the gas phase is determined mainly by the surface area density of the aerosol which, in turn, depends on the concentration and size distribution of the aerosol particles. In these conditions, a lower limit to the sulphuric acid abundance may be estimated from vapour pressure considerations assuming sulphuric acid to nucleate as a binary system (H_2SO_4–H_2O) which leads to the H_2SO_4 volume mixing ratio profile shown in Fig. 2 (ref. 12). The profile corresponds to a mean temperature profile for mid-latitude spring–autumn conditions as given by the US standard atmosphere (1966) and to a constant water vapour volume mixing ratio of 3.5 p.p.m.V.

An upper limit to the sulphuric acid abundance may be estimated from photochemical–diffusive models neglecting H_2SO_4–aerosol processes. As an example, a profile deduced from Harker's model[13] (case 1) is also shown in Fig. 2. When compared with the profile based on vapour pressure considerations, this gives higher values at heights below ~34 km, implying that H_2SO_4 nucleation in the binary system occurs below this height for the temperature profile used. Considering the lower temperature measured during our balloon experiment at 36.5 km (227 K compared with a value of 239 K as used by Hamill *et al.*[12] the H_2SO_4 vapour pressure and thus the H_2SO_4 abundance should have been considerably lower than that shown in Fig. 2. According to the data given in Table 1 of Hamill *et al.*[12], the difference may be of the order of a factor of 20.

An H_2SO_4 profile given by a recent comprehensive model which considers photochemistry, vertical transport and aerosol processes of sulphur compounds[6] is also shown in Fig. 2. This profile which is based on the same temperatures as the profile of Hamill *et al.*[12] is similar, as would be expected from a combination of the limiting profiles H 75 and HA 77 (see Fig. 2).

When compared with our experimental sulphuric acid abundance also given in Fig. 2, the model values are much too high. Considering, however, a lower H_2SO_4 vapour pressure associated with the lower temperature measured during our balloon experiment the expected H_2SO_4 volume mixing ratio at 36.5 km may have been low as about 0.15 p.p.b.V. which is much closer to the experimental value but still far too high.

Therefore, current models apparently fail to describe the abundance of stratospheric gas phase sulphuric acid. Possible causes such as an overestimate of the H_2SO_4 production or an underestimate of the H_2SO_4 loss will now be briefly investigated. The most severe uncertainties of the model computations seem to be associated with (1) poorly known natural fluctuations of the vertical transport of COS strongly affecting the COS abundance and thus the H_2SO_4 production around 36 km; (2) unknown photoabsorption coefficients for H_2SO_4 which were simply assumed to be the same as those of HCl (ref. 6); (3) considerable uncertainties of H_2SO_4 vapour pressure computations[14,15]. Considering a discrepancy between experimental and model H_2SO_4 abundances of about a factor of 100 it seems that each of the above uncertainties may be sufficient to account for the disrepancy. Other processes which were not included in the models may be responsible. One is H_2SO_4 nucleation in the hypothetical three-component system (H_2SO_4–HNO_3–H_2O) which is suspected to have a much lower H_2SO_4 vapour pressure than the two-compoent system (H_2SO_4–H_2O)[16]. Whether or not H_2SO_4 nucleation can account for the low sulphuric acid abundance observed, may be judged from a comparison of H_2SO_4 lifetimes in the gas phase. The lifetime for nucleation t_N is of the order of 3×10^5 at 36.5 km if a typical nuclei concentration of $10\,cm^{-3}$ and a typical nuclei size of 0.04 μm (ref. 17) are considered. For comparison, the lifetime t which can be derived from the experimental sulphuric acid concentration using an H_2SO_4 production rate of about $0.6\,cm^{-3}\,s^{-1}$ at 36.5 km (deduced from the model of Turco *et al.*[6]) is about 3×10^5 s. The agreement of t and t_N suggests that at least as far as time constants are concerned the low H_2SO_4 abundance observed is consistent with an efficient removal of gas phase H_2SO_4 by nucleation. The critical question is whether H_2SO_4 was at all supersaturated and thus able to nucleate.

An alternative sink for gas-phase H_2SO_4 may be reactions with negative ions followed by the formation of stable ion pairs which ultimately become incorporated into aerosol particles[18]. The H_2SO_4 lifetime for this process may be as low as about 5×10^5 at 36.5 km which is close to the inferred t.

The cause of the discrepancy between experimental and theoretical abundances of gas phase sulphuric acid in the stratosphere can not be identified with certainty. Further *in situ* measurements of H_2SO_4 abundances need to be extended in height and they should be carried out in conditions of different stratospheric temperatures. Particularly in summer, when the mean temperature around 36 km may be as high as about 252 K in middle latitudes, the vapour pressure of H_2SO_4 should be higher than during our present experiment (227 K) by 2 or 3 orders of magnitude. If such an increase was observed H_2SO_4 nucleation could be identified as the cause in question.

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Stratospheric observation of far IR pure rotational lines of hydroxyl

D. J. W. Kendall

Bomem Inc, 910 Place Dufour, Ville de Vanier, Quebec,
Canada GIM 3B1

T. A. Clark

Department of Physics, University of Calgary, Calgary, Alberta,
Canada T2N 1N4

The hydroxyl radical, OH, has a key role in many atmospheric and ionospheric processes and has been detected in molecular clouds in space. The well-known near IR vibration–rotation bands of this molecule, named after their discoverer, Meinel, were first identified in atmospheric observations[1] while much of the impetus for precise microwave data has come from the identification of radiation in this wavelength range from OH in space[2]. However, although increasingly precise measurements have now been reported from the UV[3] through the IR[4] to the microwave spectral regions[5], the far IR pure rotational line spectrum has been virtually ignored, and no observations of this relatively simple spectrum have been reported between 5 and 500 cm^{-1}. Here we present measurements of spectra between 30 and 110 cm^{-1} and deduce stratospheric OH column densities from the observed spectral lines.

We have recalculated[6] the expected pure rotational line positions for all vibrational levels up to and including $v = 9$ for the ground electronic $^2\pi$ state and have derived values for the integrated intensity ($cm^{-2}\,atm^{-1}$) for these lines in the ground electronic and vibrational state at a temperature of 220 K, corresponding to a mean stratospheric temperature. This study showed that the integrated intensity values were very large and suggested that pure rotational lines would be detectable in far IR emission spectra taken in the stratosphere from a balloon platform.

This prediction is important as the amount of hydroxyl in the stratosphere has become an important, even vital, parameter in the current debate on the possible modification of the ozone layer by halocarbons, and measurements of this radical are urgently needed for inclusion in models of ozone behaviour[7]. Only one group has reported direct measurements of OH in the stratosphere[8], and values obtained by a balloon-borne *in situ* sampling technique, in conjunction with a ground-based resonance fluorescence measurement[9] have shown that the stratospheric OH concentration apparently exhibits large daytime variations. Recent theoretical calculations have shown that while the greatest concentration of OH occurs in the mesosphere at 80 km at night, this peak in the OH density profile moves to the upper stratosphere at 40 km during the daytime[10,11].

The predicted line strengths for pure rotational lines of OH led to the careful examination of emission spectra of the stratosphere taken during a balloon flight series in 1976 from Yorkton, Saskatchewan, Canada (51.3° N, 102.3° W). On one of these flights on 15–16 August, 1979, a simple rapid-scanning Michelson interferometer[12] measured a series of high resolution (0.05 cm^{-1}) spectra between 30 and 110 cm^{-1} of the stratosphere, at an angle of 84.2°, from an altitude of 24.5 km during the evening and sunset periods. An average of 15 of these spectra has been obtained, selected sections of which are presented in the upper parts of Fig. 1*a–c*. The low altitude and high zenith angle conditions combined to produce very strong H_2O emissions and to enhance weak O_3 lines. In these spectral regions, the predicted positions for the Λ-type doublet OH lines are indicated. Figure 1*c* shows a predicted H_2O and O_3 spectrum, produced from the AFGL line listing[13]. The present

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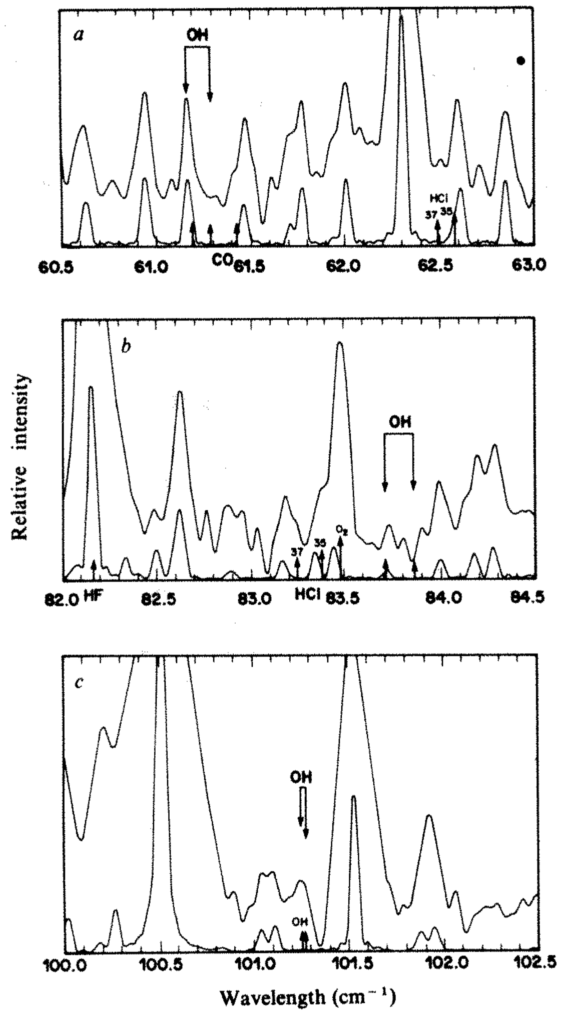

Fig. 1 *a–c*, Sections of the stratospheric far IR emission spectrum, observed at a mean altitude of 24.5 km at a zenith angle of 84.2°. These sections are the sum of 15 unapodised spectra computed at a resolution of 0.05 cm^{-1}. An equivalent synthetic spectrum for H_2O and O_3 to a resolution of 0.05 cm^{-1} is shown below each section.

spectra are somewhat better than those previously reported from a different flight[6] in having higher resolution and better signal-to-noise ratio in the spectral data. As can be seen from Fig. 1, the OH line positions for the ground electronic and vibrational state are in spectral regions which are relatively free of other lines, and, even though some blending with weak O_3 lines occurs, and other small features in the spectrum are at present unassigned, a tentative identification of pure rotational OH emissions can be made. The lines at 61.29 cm^{-1} (with an observed feature at 61.30 cm^{-1}), at 83.85 cm^{-1} (observed feature at 83.90 cm^{-1}) and the combined and strong feature at 101.26 cm^{-1} can be used along with the predicted line strengths[6] to yield an average column density of OH of $8(\pm 3)\times 10^{13}$ molecules cm^{-2}. Assumption of a constant mixing ratio above the altitude of 24.5 km yields a value of about 0.1 parts per 10^9 by volume for this mixing ratio, a value which is in reasonable agreement with the values obtained by Burnett[9].

Thus, even this rather tentative identification is encouraging in indicating that the technique may prove particularly valuable for monitoring this important constituent in the stratosphere, at least during the daytime. Emissions from the higher layers during night-time hours would perhaps make interpretation of the data difficult, although such results might be of interest in their own right for other scientific studies. Higher resolution and sensitivity in the instrumentation during future observations should improve the identification of these pure rotational OH lines, and angular scanning of the instrument across the atmospheric limb to high zenith angles should allow a profile of OH density to be obtained, a parameter which would be of great value in ozone layer modelling. There is also an urgent need for laboratory measurements to verify the position and integrated intensity values for these pure rotational far IR transitions.

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Methyl chloride in the stratosphere

S. A. Penkett, R. G. Derwent

Environmental and Medical Sciences Division, AERE, Harwell, UK

P. Fabian, R. Borchers

Max-Planck Institut für Aeronomie, Katlenburg/Lindau, FRG

U. Schmidt

Institut für Atmospharische Chemie, der Kernforschungsanlage Julich GmbH, Julich, FRG

There is much current interest in the chemistry of the stratosphere and the possible impact of chlorine compounds on the Earth's ozone layer. Profiles of anthropogenic compounds such as $CFCl_3$ and CF_2Cl_2, which are emitted from aerosol spray cans, have already been measured in the stratosphere[1]. They confirm that substantial photolysis of these compounds is occurring and thereby releasing chlorine atoms which can catalyse ozone removal. The only natural chlorine containing compound which is likely to enter the stratosphere at significant concentrations is methyl chloride. It is therefore important that its profile be measured and compared with a computed profile to ascertain the likely impact on the ozone layer of naturally occurring chlorine. We report here measurements of the methyl chloride concentration profile up to an altitude of 32 km. The concentration falls off with altitude due to reaction with hydroxyl radicals and it confirms that chlorine of anthropogenic origin is now predominant in the stratosphere.

Lovelock[2] first reported the presence of methyl chloride in the atmosphere at the 1–2 p.p.b. level (1 p.p.b. = 1 part in 10^9 by volume) and showed that its concentrations were highest in air whose origins were oceanic rather than continental. Measurements in seawater confirmed that the ocean could indeed be a significant source in addition to that produced by the smouldering combustion of vegetation. Subsequently studies have provided considerable information on the tropospheric distribution of methyl chloride. Singh *et al.*[3] report mean concentrations of 611 ± 84 p.p.t. (1 p.p.t. = 1 part in 10^{12} by volume) and 615 ± 103 p.p.t. in the Northern and Southern Hemispheres, respectively. They find evidence for a large, natural oceanic source and estimate a global source strength of 3×10^{12} g yr^{-1}. Rasmussen *et al.*[4–6] find similar values in both hemispheres, with higher values in the atmospheric mixing layer over oceans, strongly indicating an oceanic source. Additionally, they have given

Table 1 Measurements of methyl chloride in the stratosphere during June 1978 at 44° N

Probe no.	Altitude	Latitude	MeCl (p.p.t.)	Comments
1	32.6 km	44° N	6.6 8.5	Repeat analysis
2	31.3 km	44° N	57.5	
3	28.4 km	44° N	62.3 59.4	Repeats, also 50/52 ratio taken
6	19.7 km	44° N	375	
7	17.5 km	44° N	476	50/52 ratio taken.
	0.1 km	51° N	590 651 759	Harwell ground level samples

Methyl chloride levels are given in parts per 10^{12} in air by volume. Measurements at Harwell were made on the day of analysis (20 March 1979).

some attention to Lovelock's suggestion concerning 'slash and burn' agriculture. Elevated concentrations are clearly evident in Kenya and concentrations up to 2 p.p.b. have been reported[7]. In view of the widespread nature of this agricultural practice in tropical latitudes[8], this source may be of some significance when it is appreciated that much of the transport to the stratosphere takes place through the tropical tropopause[9]. The importance of this source for the stratosphere may not necessarily be reflected in the relative source strengths (3×10^{12} g yr^{-1} for the natural oceanic[2] and 3–6 × 10^{11} g yr^{-1} from the combustion of vegetation)[10]. A measured altitude profile of methyl chloride would certainly help resolve the magnitude of the stratospheric chlorine injection and the relative contributions from the ocean and combustion sources.

To measure methyl chloride in the stratosphere we utilised a balloon probe which collected 10-l samples of air at STP at eight altitudes during the descent phase of the balloon between 32.6 and 17.5 km. The samples were taken over France at a latitude of 44° N. They were cryotrapped into reservoirs contained in the probe using liquid neon and precautions were taken to avoid contamination from the balloon assembly[1]. The air samples in the reservoirs were subsequently transferred into 600 ml stainless steel flasks fitted with metal bellows valves. The pressure in the flasks was typically 4 bar at the time of analysis. Air from the flasks was analysed for a number of components using a gas chromatograph/mass spectrometer combination (Hewlett Packard 5710 GC/VG Micromass 16F MS). Methyl chloride was separated on a 3-m OV 101 column at 20 °C and was measured by single ion monitoring at mass 50. The ratio of the signals from mass 50 to mass 52 was measured in two of the stratospheric samples and it compared well with that expected for methyl chloride. About 100 ml of air was used for each analysis and the signal-to-noise ratio was such that 5 p.p.t. could be measured with ±50% accuracy.

The results for a flight in June 1978 are given in Table 1, and the altitude profile in Fig. 1. Also shown in Table 1 are measurements made at ground level at Harwell on the day of analyses. These agree well with previous determinations of methyl chloride in the troposphere[3,6], and confirm that little or no decay had occurred in the containers between flight time and analysis. Unfortunately no data are available between 28 and 20 km because of a malfunction in the balloon probe. In consequence, data are available for only five altitudes, but these show a regular decrease with height in good agreement with our calculated profile. This calculated profile was obtained using a one-dimensional model described elsewhere[11]. This calculation utilised available rate coefficients for the $OH + CH_3Cl$ reaction[12,13], UV absorption cross sections for CH_3Cl (ref. 14) and a lower boundary concentration of 600 p.p.t. at 12 km established on the basis of aircraft measurements[4,5].

Table 2 Contributions to the stratospheric chlorine budgets from the various halocarbons

Halocarbon	Chlorine contribution p.p.b.	Comment	Refs
Methyl chloride	0.7	Natural	This work
Carbon tetrachloride	0.5	Man-made	3, 19
CCl_3F	0.4	Man-made	3, 19, 20
CCl_2F_2	0.4	Man-made	3, 20
Other organic chlorine compounds*	0.6	Man-made	21
Total	2.6		

* CH_3CCl_3, CHF_2Cl, C_2Cl_4, $CHCl_3$, CH_2Cl_2, C_2HCl_3.

The close agreement between calculation and observation is typical of the behaviour found[11] for other trace gases whose sources are widely distributed throughout the troposphere. This suggests that methyl chloride is predominantly oceanic in origin. A substantial tropical injection from 'slash and burn' agriculture would result in considerable deviation between the observed profile (at 44° N) and the calculated profile (for 30° N) in the lower stratosphere due to polewards transport by the mean circulation and large-scale eddies. In the absence of a significant additional tropical injection, published values of the tropospheric levels of halocarbons can be used to estimate the likely contribution from natural and man-made compounds to the chlorine content of the stratosphere. Table 2 shows that the chlorine from natural methyl chloride is heavily outweighed by chlorine from man-made sources.

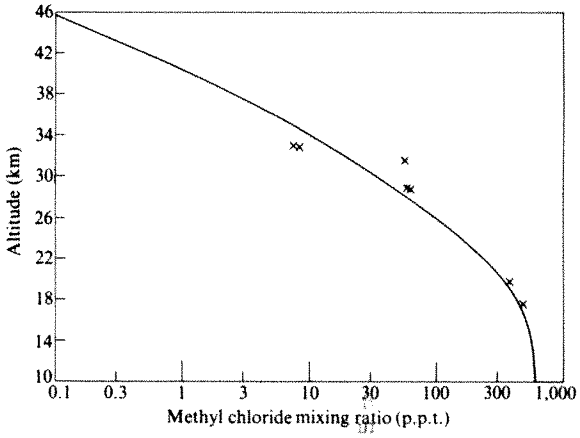

Fig. 1 Observed and calculated altitude profiles of methyl chloride. X, Measurements at 44° N during June 1978; line, calculated with 1-D model[11] using the K_z profile of Chang[22].

Finally it should be noted that the maximum amount of chlorine which can be expected from compounds presently known (2.6 p.p.b.) is substantially lower than the chlorine measured in the form of HCl and ClO in the stratosphere. Recent measurements give values of HCl up to 2 p.p.b. (refs 15, 16) and ClO between 2–8 p.p.b. (refs 17, 18) pointing to an apparent discrepancy which is beyond the scope of the present letter to discuss in detail.

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Two types of clinoenstatite in Indarch enstatite chondrite

C. A. Leitch & J. V. Smith

Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

Enstatite chondrites are characterised by highly reduced minerals[1,2]. Clinoenstatite and rare olivine are almost Fe-free, carbon occurs as graphite, and Fe, Ni-rich metal contains up to 4% Si. Ca, Ti, Mg and Mn, normally incorporated solely in silicates, are distributed between silicates and sulphides; Ti may also occur as a nitride. We now describe two types of clinoenstatite in the Indarch enstatite chondrite, one luminescing blue and one red. These clinoenstatites have distinct chemical compositions which indicate different redox conditions. The Indarch chondrite is an unannealed mechanical aggregate of diverse materials, and cannot have formed by simple progressive condensation of the solar nebula.

The Indarch chondrite contains four chondrule types[3]: porphyritic (most abundant), fine-grained, radiating, and barred. Although the minerals of the chondrules appear homogeneous in a petrographic microscope, a cathodoluminescence microscope revealed distinct red and blue types of clinoenstatite crystals. Complex changes of intensity of luminescence occur across some grains, but no chemical variation was found by electron microprobe analysis. Perhaps the intensity change results from crystallographic factors dependent on orientation of twinned and disordered regions. (The polymorphic type of pyroxene was not identified in the polished section but is assumed to be clinoenstatite following ref. 2). Each porphyritic chondrule contains both blue and red crystals but with a strong majority of either one or the other. Radiating chondrules contain large near-euhedral crystals of blue clinoenstatite surrounded by a radiating matrix of red clinoenstatite and other minerals (Fig. 1), perhaps resulting from devitrification. Fine-grained chondrules contain blue clinoenstatite except for a thin rim of red clinoenstatite found in one chondrule. All four types of chondrules are surrounded by a shell of fine-grained material composed of all mineral types found in the meteorite. A shell also occurs around chondrules in some ordinary chondrites[4].

Electron microprobe analyses (Table 1) were obtained with an ARL-EMX instrument. Mg, Si and Fe were determined with a solid-state detector, 15 kV, 0.1 μA beam current, 120 s live time. Synthetic $MgSiO_3$ glass was the standard for Mg and Si, and P-140 olivine for Fe. Minor elements were determined with spectrometers at 25 kV and 2 μA. Counting times for peak and background were chosen to give a precision near 30 p.p.m. (1σ) for an individual analysis; precision of mean values is lower. Standards were $Di_{85}Jd_{15}$ glass (Ca, Na, Al), $Ca_2P_2O_7$(P), Corning glass V (Ti, Cr), Corning glass W(Mn) and Corning glass X(Ni). Secondary fluorescence could contribute but is significant only for Fe analyses made on clinoenstatite lying close (<0.1 mm) to metal or troilite. Analyses deliberately made on clinoenstatite crystals distant from metal and troilite varied only about 10% in Fe whatever the colour of the clinoenstatite and the type of host chondrule, but random analyses showed some higher values of Fe. The slightly high totals (100.5–101.6) probably result from a small change of thickness of carbon coat between the standard and the unknown; such a small change would have only a trivial effect on atomic ratios.

The minor elements correlate with the luminescence colour but not with the chondrule type. Whereas the red clinoenstatites have higher concentrations of Ti, Al, Cr, Mn and Ca, the blue ones tend to have higher Na. Assignment of valence states to minor elements in pyroxenes depends on crystal-chemical theory, and the following assignments follow currently accepted practice based on ionic potentials.

For blue clinoenstatite, P was assigned to $NaMgSiPO_6$, Ti to $NaTiAlSiO_6$, Al to $NaAlSi_2O_6$ and Cr to $NaCr^{3+}Si_2O_6$. The remaining Na could not be accounted for except by assuming a corresponding amount of trivalent iron in the acmite unit $NaFe^{3+}Si_2O_6$, or the alternative of vacant sites. Based on 24 oxygen atoms per cell, 0.004–0.017 Fe^{3+} atom was needed. The remaining divalent species were coupled with Si. For a perfect analysis, there should be eight Si atoms per cell, apart from the trivial deviation caused by the insignificant amounts of P and Ti, and indeed the observed values of Si lie within experimental error of eight atoms.

For red clinoenstatites, P was assigned to $NaMgSiPO_6$, Ti to $NaTiAlSiO_6$ and the remaining Na or Ti to either $NaAlSi_2O_6$ or $MgTiAl_2O_6$. Some Al and Cr was still unused, and it was necessary to use $MgAl_2SiO_6$ and $MgCr^{3+}{}_2SiO_6$ units. Furthermore, the sum of the remaining divalent ions (assuming all Fe as Fe^{2+}) was less than the number of Si^{4+} ions. Because the blue clinoenstatites calculated closely to an MTO_3 formula, this discrepancy was not attributed to experimental error, and about one-third of the Fe was converted to the trivalent state. A similar conclusion with slightly different numbers was reached more directly by adjusting the oxygen/metal ratio to the theoretical value of 3:2 by converting some Fe to the trivalent state.

Note that our suggestion of the presence of Fe^{3+} is questionable because it depends on crystal-chemical assumptions and analytical error. Direct spectral evidence is needed to confirm or refute the suggestion. A wet chemical analysis for a pyroxene separate from the Kota-Kota enstatite chondrite[5] showed a deficiency of Si, 0.93 wt % FeO, and only 0.03% Fe_2O_3. For the present, we note that even if there is no Fe^{3+} in the Indarch clinoenstatites, the unequal content of Ti, Cr, and Mn in the red

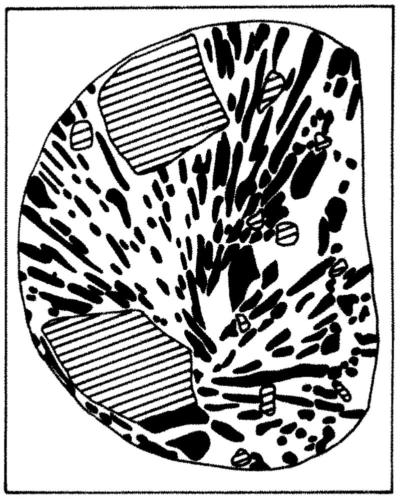

Fig. 1 Schematic representation of a fragment of a radiating chondrule. Black areas are red clinoenstatite; hatched regions are blue grains; clear areas are composed of glass or very fine-grained sulphides. The maximum width of the chondrule is 600 μm. Thin section from Field Museum collection.

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Table 1 Analyses of clinoenstatites

	Indarch						Kota–Kota
		blue			red		
	1	2	3	4	5	6	7
P_2O_5	0.003	0.002	0.001	0.002	0.005	0.002	<0.1
SiO_2	59.9	59.8	59.9	60.1	60.0	59.7	58.2
TiO_2	0.002	0.002	0.005	0.059	0.052	0.027	0.03
Al_2O_3	0.051	0.057	0.038	0.267	0.293	0.147	0.32
Cr_2O_3	0.028	0.043	0.053	0.259	0.339	0.385	0.36
FeO_T	0.95	0.89	0.94	0.94	0.93	0.86	(0.96)
MnO	0.067	0.070	0.030	0.274	0.115	0.101	0.1
MgO	39.4	39.5	39.6	39.6	39.5	39.7	39.5
NiO	0.005	0.003	0.004	0.011	0.003	0.006	0.034
CaO	0.072	0.054	0.058	0.200	0.254	0.143	0.4
Na_2O	0.109	0.068	0.095	0.041	0.062	0.005	0.05
Total	100.587	100.489	100.724	101.753	101.553	101.076	100.0
cations/24 oxygens							
P	0.0003	0.0002	0.0001	0.0002	0.0006	0.0002	—
Si	7.9977	7.9910	7.9847	7.9463	7.9468	7.9375	7.864
Ti	0.0002	0.0002	0.0005	0.0059	0.0052	0.0027	0.004
Al	0.0080	0.0090	0.0060	0.0416	0.0458	0.0231	0.052
Cr^{3+}	0.0030	0.0045	0.0056	0.0271	0.0355	0.0405	0.040
Fe^{3+}	0.0166	0.0041	0.0130	0.0370	0.0288	0.0568	—
Fe^{2+}	0.0893	0.0953	0.0920	0.0670	0.0743	0.0391	0.108
Mn	0.0076	0.0079	0.0034	0.0307	0.0129	0.0114	0.012
Mg	7.8410	7.8680	7.8680	7.8044	7.7979	7.8666	7.948
Ni	0.0005	0.0003	0.0004	0.0012	0.0003	0.0006	0.004
Ca	0.0103	0.0077	0.0083	0.0284	0.0361	0.0204	0.056
Na	0.0277	0.0176	0.0246	0.0105	0.0159	0.0013	0.012
Total	16.0022	16.0058	16.0066	16.0003	16.0001	16.0002	16.100

Columns 1–6, Electron microprobe analyses; iron given as FeO. Column 1, Mean of six analyses of blue grains in predominantly red porphyritic chondrule. 2, Mean of six analyses of blue grains in predominantly blue porphyritic chondrule. 3, Mean of five analyses of large blue crystals surrounded by red matrix of radiating chondrule. 4, Mean of four analyses of red grains in predominantly red porphyritic chondrule. 5, Mean of five analyses of red grains in predominantly blue porphyritic chondrule. 6, Mean of six analyses of red matrix of radiating chondrule. 7, Microchemical analyses of bulk pyroxene separate[4]; FeO 0.93, Fe_2O_3 0.03 wt%; also K_2O 0.005.

and blue clinoenstatites must indicate different redox conditions if both clinoenstatites equilibrated with sulphide.

Mechanical aggregation of diverse source materials is required to explain the complex texture and bimodal chemistry of the clinoenstatite. Direct derivation from hypothetical progressive condensation of a gaseous solar nebula can be ruled out, and a complex origin is needed, perhaps involving a rapidly cooled cloud of solid particles, liquid droplets and gas undergoing aerodynamic sorting[6] coupled with settling onto the surface of a growing planetesimal. Perhaps the cloud formed from collision between two planetesimals[7]. Petrographic and mineralogical evidence is accumulating for the presence of assemblages in chemical disequilibrium in all types of unequilibrated chondrites (as reviewed in ref. 7, and emphasised in ref. 8).

Absence of a reaction zone between adjacent blue and red clinoenstatites rules out prolonged annealing of the Indarch enstatite chondrite. If the clinoenstatites really do contain some trivalent iron, they could not be in chemical equilibrium with Si-containing Fe,Ni metal, and mechanical aggregation from two distinct source regions would be implied. Indeed the clinoenstatite-bearing chondrules do not contain large fragments of metal. Tiny grains of metal, too small for routine analysis with an electron microprobe, occur in the porphyritic chondrules, but are tentatively attributed to mechanical aggregation and vapour deposition during coalescence of the red and blue clinoenstatites.

There is undoubted petrographic and chemical evidence of chemical disequilibrium in the Indarch enstatite chondrite. The implied presence of trivalent iron needs testing by spectral techniques, and detailed analyses of trace elements are needed for all minerals. Preliminary study of the Abee, Kota-Kota and Adhi-Kot enstatite chondrites has revealed both red and blue enstatites, and electron microprobe analyses are in progress.

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Gravity anomalies, block-faulting and Andean-type tectonism in the Eastern Churchill Province

M. D. Thomas

Gravity and Geodynamics Division, Earth Physics Branch, Department of Energy, Mines and Resources, Ottawa, Canada K1A OY3

P. Kearey

Department of Geology, Queen's Building, University Walk, Bristol, UK

Evaluation of the tectonic evolution of Precambrian terrains may be greatly enhanced by selecting appropriate analogues from Phanerozoic examples where the geological history is better understood and where a knowledge of current geodynamic processes is sometimes available. The Churchill Structural Province in the eastern Canadian Shield apparently has a close analogue in the central Andes of Peru where the Nazca plate continues to thrust below the South American plate. We propose that this provides the basis for a tectonic framework that explains several geological features both within the Churchill Province and at the adjacent boundaries with the Superior and Nain provinces.

The Churchill Structural Province in northeastern Quebec and Labrador is characterised by a distinctive pattern of coextensive, parallel, regional gravity anomalies extending laterally 300–450 km and having widths between 45 and 100 km. These anomalies strike approximately north-northwest and parallel large-scale features, such as the boundaries of the Churchill Province with the Superior Province to the west and the Nain Province to the east, certain major lithological units (Fig. 1), and structural trends apparent from field data and air photographs[1].

The negative–positive pair of anomalies *a* and *b* (Fig. 1) have been related to structures formed by the collision of Superior and Churchill protocontinents during the Hudsonian Orogeny[2] following subduction of the Superior-bearing plate beneath the Churchill Province. The structures are consistent with a 'basement reactivation' model[3], in the context of which negative anomaly *a* was explained by downwarped Superior crust and positive anomaly *b* by topography on the Conrad discontinuity, developed during collision, within the thickened Churchill crust[2]. The same dipolar gravity signature is common to several of the structural province boundaries in the Canadian Shield[4]. The type structure of such boundaries, interpreted as sutures, consists of an older crustal block, that thickens towards the suture, in steep contact with a relatively thicker and denser, younger block, both blocks being in approximate isostatic equilibrium.

Suggested causes of gravity anomalies east of the boundary pair are topography on the Conrad discontinuity[2,5] and, in some cases, lithological variations[5]. However, there are several lines of evidence, including the extensive nature of the gravity anomalies themselves, to indicate that these regional anomalies are related to major, fault-bounded crustal blocks which are laterally extensive, of differing density and in relative isostatic equilibrium[6] (see model, Fig. 2*A*); previous interpretations[2,5]

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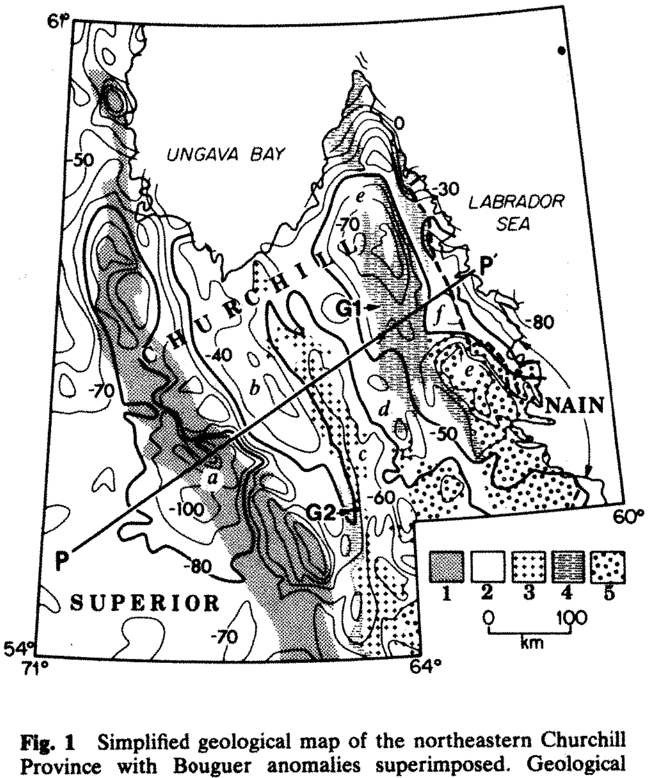

Fig. 1 Simplified geological map of the northeastern Churchill Province with Bouguer anomalies superimposed. Geological legend for Churchill Province: 1, Labrador Trough; 2, mixed gneisses and migmatites; 3, granites and granodiorites; 4, granulites; 5, anorthosites and adamellites. G1 and G2 indicate areas of granulite. The Churchill–Nain boundary is identified by bold dashed line. Bouguer anomalies are reduced to sea level using a uniform density of 2.67 g cm^{-3} and are contoured at an interval of 10 mGal. Bold contours outline anomalies (*a–f*) discussed in text. P–P′ is profile interpreted in Fig. 2*A*.

are not necessarily invalidated by this hypothesis but rather become a matter of detail. Geological evidence for all of the large-scale faults interpreted in the model is incomplete, perhaps due to the reconnaissance nature of the mapping and the considerable cover of glacial drift[7,8]. Nevertheless, a 200-km long fault zone has been identified close to the gradient separating anomalies *e* and *f* where it forms much of the eastern boundary of a large area of granulites (G1, Fig. 1). Faults have also been mapped[9,10] along 100 km of the western border of granulites (G2) which correlate with the southern part of positive anomaly *b*. Other prominent faults in the region have north and northwesterly strikes and are indicated by zones of mylonite[7,8]. Relative vertical movements of crustal blocks are indicated by the juxtaposition of high and low grade metamorphic rocks: amphibolite facies terrain to the east of the Labrador Trough underlying anomaly *b* has probably risen 10–15 km relative to lower grade terrain to the west[11]. The eastern limit of this uplifted terrain may coincide with a change in the pattern of aeromagnetic anomalies that occurs roughly along the −40 mGal contour on the east flank of gravity anomaly *b* (Fig. 3) and is considered further evidence in support of a zone of major faulting between blocks underlying anomalies *b* and *c* (Fig. 2*A*).

The gravity model of the Churchill crust (Fig. 2*A*) is comparable in morphology and scale to a crustal model of the Andes proposed for central Peru[12] (Fig. 2*B*). Development of the latter model over the past 100 Myr has been influenced by large-scale vertical faults[12–14] parallel to the Peru–Chile trench that may result from strong coupling between the Nazca and South American plates[15]. Movement along these faults has controlled the following aspects of Andean geology: basin development, batholith emplacement, and the style of deformation of basin fill[12,14]. Basin development and batholith emplacement in the Churchill Province likewise seem to have been controlled by major faults, but any comment regarding deformational styles is precluded by a lack of detailed structural information and the high metamorphic grades. The remains of a former major sedimentary basin, the Dorset Fold Belt[16], have been identified in the region of negative gravity anomaly *e* (Fig. 1) and continue into Baffin Island. Its Andean analogue is the East Peruvian

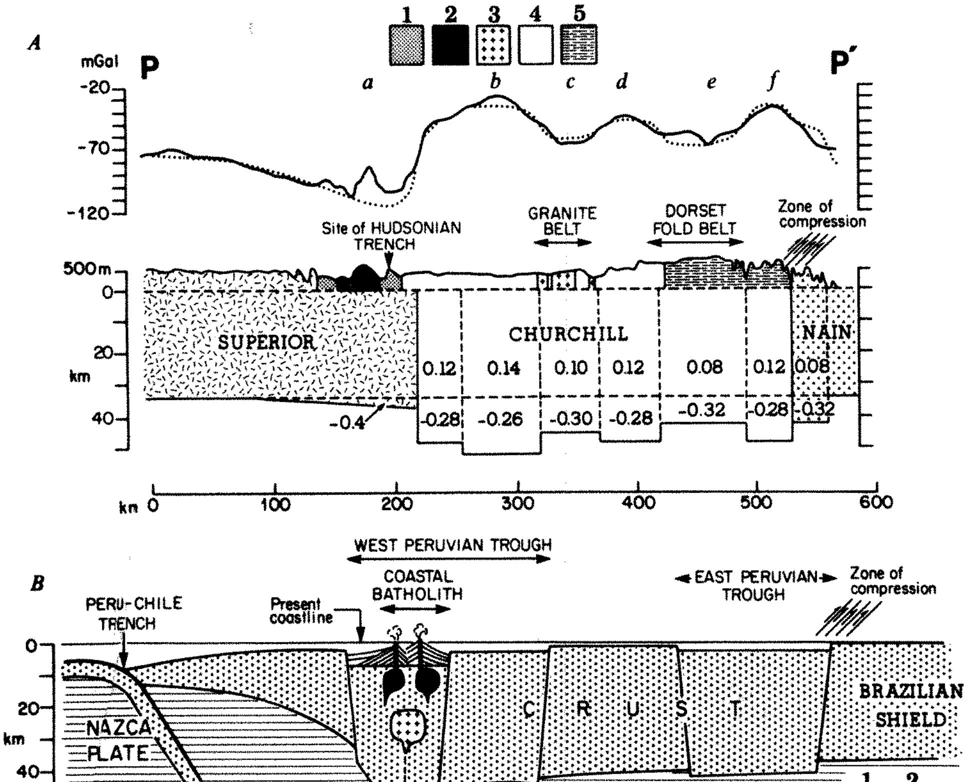

Fig. 2 *A*, Interpretation of gravity profile P–P′ with Churchill Province modelled as several blocks, each in relative isostatic equilibrium. Solid line, observed gravity; dotted line, calculated gravity. Letters *a–f* identify anomalies discussed in text. Geological legend: 1, metasediments of the Labrador Trough; 2, basic igneous rocks of the Labrador Trough; 3, granitic rocks; 4, mixed gneisses and migmatites; 5, granulites. Density contrasts in g cm^{-3}. *B*, Schematic interpretation of a crustal section across the central Andes at 100 Myr ago (after Myers[14]). Geological legend: 1, basic intrusions; 2, the rising Coastal Batholith.

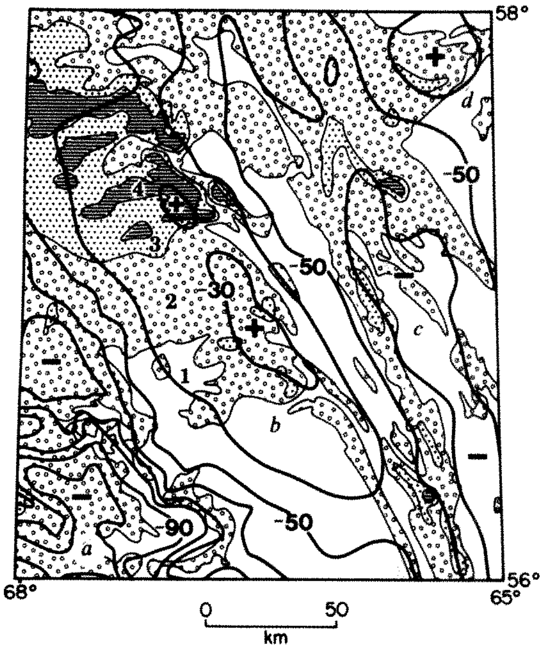

Fig. 3 Portion of Churchill Province adjoining the Labrador Trough for which there are published aeromagnetic data. Aeromagnetic anomalies are contoured at 400 nT intervals and increase in intensity from pattern 1 to pattern 4. Superimposed Bouguer anomalies are contoured at 10 mGal interval and outline parts of anomalies *a*, *b*, *c* and *d* (Fig. 1). A change in the crustal character is evidenced in the aeromagnetic anomalies approximately along the line of the −40 mGal Bouguer anomaly contour which lies along the gradient between anomalies *b* and *c*.

Trough (Fig. 2*B*). Negative gravity anomaly *c* (Fig. 1) coincides with an almost continuous belt of granitic rocks 500-km long. The restriction of granitic rocks to a narrow, linear belt suggests that they have their analogue in the Andean Coastal Batholith which may have been localised by major shear zones and was formed from plutons and sheets intruded into the same tabular belt by repeated cauldron subsidence[12]. Further, the noted absence of syn- and post-orogenic granitic intrusions within the Circum-Ungava belt[17,18] could be explained if the Churchill granite had a similar spatial relationship to the interacting Superior and Churchill plates as the coastal batholith had to the Nazca and South American plates. By this analogy, no granites would be expected within the Labrador Trough, here interpreted as the approximate site of the Hudsonian trench. Rather, granites would be expected some 230 km east of the trough; they are, in fact, centred 70–150 km from the trough (Fig. 1). The smaller distances, most probably, are a result of crustal shortening associated with collision, although the angle of subduction also may have been a contributing factor. Shortening of the Labrador Trough deposits, by thrust faulting and folding, is estimated[19] to have been at least 113 km. The Churchill basement to the east also bears evidence of shortening in the form of tightly pinched synclines, many overturned towards the Superior Province[18]. Radiometric ages (K–Ar) on the Churchill granitic rocks[7] of 1,580 Myr[20] and 1,595 Myr[21] indicate that they were intruded during the late stages of the Hudsonian Orogeny.

According to our model the Churchill–Nain boundary represents the eastern limit of crustal reactivation related to subduction of an oceanic plate, bearing the Superior Province, below an Archean protocontinent comprising what are now the Churchill Province and the North Atlantic Archean Craton[22]. Above the subducted plate, reactivation processes—manifest as the Hudsonian Orogeny—have reset the radiometric clock and transformed Archean crust to Proterozoic crust at the same time imparting to it structural characteristics which distinguish the region as the Churchill Province. The Nain Province, a segment of the now dissected North Atlantic Archean Craton, remained unaffected. Detailed mapping[23] at the Churchill–Nain boundary has revealed evidence of crustal compression in the form of west dipping thrusts and axial planes of folds attributed to the Hudsonian Orogeny. The structures at this boundary have their counterparts in the Andes where similar compressive tectonics occur in the sub-Andean ranges[24] centred some 500–700 km east of the Peru–Chile Trench. Here, the crust has been compressively deformed from the west against the Brazilian Shield along a belt that marks the eastward limit of intraplate deformation associated with subduction of the Nazca Plate[25,26] (Fig. 2*B*). There has been recent speculation that the flat geometry of the Nazca Plate, dipping at about 10°, may be responsible for the broad zone of deformation across the central and northern Peruvian Andes[15]. This raises the possibility that the Superior-bearing oceanic plate also subducted at a shallow angle.

Viewed in the light of an Andean analogue several diverse features of the eastern Canadian Shield can be related to a single Proterozoic geodynamic system involving the interaction of two converging plates. Collision tectonics seem to dominate in the region within and bordering the Labrador Trough, but further east it is likely that the geology has evolved in response to the underthrusting of a Superior-bearing oceanic plate.

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Recrystallisation and electrical behaviour of glacier ice

J. M. Reynolds & J. G. Paren

British Antarctic Survey, Natural Environment Research Council,
Madingley Road, Cambridge, UK

Electrical d.c. resistivity measurements made on the George VI Ice Shelf have established the polar nature of Antarctic ice despite saturation by melt-water from surface melt-lakes. Previously it has been suggested that different impurity concentrations are responsible for the extremes in resistivity (a factor of 1,000) between temperate ice (essentially at its pressure melting point) and polar ice (well below its pressure melting point). However, the evidence suggests that impurity levels are similar despite the leaching of contaminants by free water in temperate glaciers, a process which is absent in true polar glaciers. This implies that, although impurities can significantly alter the electrical behaviour of glacier ice, there is a more fundamental mechanism at work. We suggest that this process is one of recrystallisation.

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Georesistivity measuring techniques have been applied to the sounding of ice masses many times during the last 25 years. Studies on European glaciers indicate that temperate ice has a resistivity of about 10^8 Ωm (refs 1–6). Mono- and polycrystalline ice grown slowly from pure water in the laboratory has given similar values of 2×10^6 to 5×10^8 Ωm (refs 7, 8). Experiments carried out on ice from polar regions which is well below its pressure melting point have indicated that its resistivity is three orders of magnitude smaller than that of temperate glacier ice, typically between 5×10^4 and 1.5×10^5 Ωm (refs 9–15), and that the resistivity is temperature and density dependent[10,16]. Electrical experiments undertaken in the laboratory[17,18] on cores taken from both temperate and polar glaciers have confirmed the extreme differences with respect to *in situ* measurements. Recent work has sought an explanation for this marked diversity in resistivity.

Previous *in situ* measurements have been made in the dry-snow and percolation zones of polar ice masses[1–6,12] (as defined by Müller[19]) and in the ablation zone of temperate glaciers[9–15,20]. It was with this background that a comprehensive resistivity survey was carried out on George VI Ice Shelf in the Antarctic Peninsula (71°58′ S, 67°34′ W) during January and February 1979. This particular ice shelf undergoes extensive surface melting during the summer and melt-lakes are formed which saturate the firn although the mean annual temperature for the area is −11 °C[21]. There are two basic layers in the ice shelf. At least the top 25 m of the ice shelf consists of locally accumulated snow which has been modified shortly after its deposition by free water from melt-lakes on the surface. Beneath this layer there is dense dry firn and ice from outlet glaciers flowing from the Palmer Land plateau, a cold (−20 °C)[22] dry-snow environment.

One of the aims of our study was to determine the extent to which free surface water which is subsequently frozen affects the electrical properties of ice in an area thought to be transitional percolation-soaked zone. We found that resistivities lay in the range $(5.9 \pm 1.7) \times 10^4$ Ωm, a value which is typical of cold, dry polar ice. This is an important result because it shows that the free water has affected the resistivity only indirectly. In a cold, dry region the transition from fresh snow to dense glacier ice is by a process of densification. The Ross Ice Shelf is an example of such an area and its resistivity profiles reflect a gradual increase in density with depth[13] (mean 10 m density is 0.425 Mg m^{-3}). On the George VI Ice Shelf, where the mean 10-m density is 0.832 Mg m^{-3}, our resistivity data imply that the density-depth effect is much less marked than on the Ross Ice Shelf. The water in George VI Ice Shelf melt-lakes freezes in late summer to form extensive thick ice layers of very dense ice (0.913 Mg m^{-3}) which sandwich bands of firn. The main process of densification is by percolation and refreezing and not simply by compaction. A low frequency resistivity of 10^5 Ωm was obtained for ice in a shallow lens formed from melt-water within the last 10 years. This resistivity value is comparable with those of the rest of the ice shelf. Hence, the electrical behaviour of high density ice formed on a cold glacier by compaction is very similar to that of ice formed by the percolation and freezing of melt-water. This implies that the stabilisation of the electrical behaviour of ice is completed within a few years after the ice forms even though recrystallisation continues throughout the life of the ice.

One explanation of the difference in behaviour between samples of temperate and of polar ice (and indeed, between polar ice samples of different depositional age) has been the amount of impurities (CO_2[23,24], and volcanic acids[25]) which remain in the ice after firnification. Yet soluble impurity levels in polar ice and ice in the ablation zone of temperate glaciers are similar and do not differ by orders of magnitude[23,24]. Impurity concentrations, although able to modify electrical behaviour significantly, cannot provide the basic mechanism which accounts for the difference between temperate and polar ice[26].

We wish to draw attention to the consistency of polar ice resistivities even where melt-water is present. Two potentially important mechanisms which operate in temperate glaciers and which are reduced or absent in polar ice masses are the complete removal of melt-water runoff and the extent of recrystallisation that occurs in response to stress. We now suggest that these factors may be responsible for the differences in the electrical behaviour between polar and temperate ice.

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Prediction and test of the effects of interspecific competition

Göran Högstedt

Department of Animal Ecology, University of Lund,
S-223 62 Lund, Sweden

Although interspecific competition is of major importance in ecological and evolutionary theory[1], there has been no critical study of whether indirect estimates of niche overlap accurately reflect the effects of competition on fitness as indicated by reproductive output. I report here that competition from jackdaws, *Corvus monedula*, significantly reduced reproductive output of magpies, *Pica pica*, due to starvation of nestlings and a high rate of nest predation. The competition coefficient as estimated from these direct effects ($\alpha = 0.43$) was only slightly smaller than predicted by niche overlap values based on morphological similarity, habitat distribution and feeding technique ($\alpha = 0.58$), or food composition ($\alpha = 0.54$).

The study was carried out in a south Swedish grassland habitat with scattered young pine plantations, most of them containing one pair of magpies. Niche overlap was estimated in two ways. First, the percentage overlap[2] in the frequency distribution of jackdaws and magpies foraging in different habitats (areas with heath, grassland, tall herbs and pines), and using different feeding techniques (gleaning, probing and chasing) was determined. The ratio of bill sizes in the two species was also calculated. The product of all these values gave an estimate of overall niche overlap of $\alpha = 0.58$. Second, samples of food brought by the parents to their nestlings were identified to species level, except for cereals and a few unimportant categories. The percentage overlap in frequency distribution of the food of the two species yielded $\alpha = 0.54$.

If jackdaws and magpies compete for limited resources, one would expect the effects of competition on reproductive output to be closely related to these overlap values, provided the values

Table 1 Breeding results of experimental and control pairs of magpies

	Experimental group	Control group	*P* (two-tailed)
Start of laying (median date) day 1 = 1 April	20	21	NS
Clutch size	6.50 ± 0.30	6.48 ± 0.18	NS
Mean weight of clutch	64.7 ± 4.0	67.1 ± 2.3	NS
Mean of mean nestling weight within brood, age 21–25 d	182 ± 7	208 ± 4	< 0.02*
No. of started breedings (*N*)	18	38	
No. of hatched clutches (% of *N*)	10 (56%)	23 (61%)	NS
No. of broods with ≥ 1 fledged young (% of *N*)	3 (17%)	16 (42%)	< 0.04†
No. of fledged young per successful brood	2.0 ± 0.58	4.0 ± 0.26	< 0.05*
No. of fledged young per breeding attempt	0.33 ± 0.20	1.68 ± 0.34	< 0.001

Figures are given ± s.e. NS, not significant.
* Mann-Whitney *U* test.
† Fischer exact probability test.

give an accurate measure of niche overlap. The values are directly comparable to the competition coefficient because the two species have almost identical weights and, therefore, also identical consumption rates.

This prediction was tested with a 2-yr experiment. Three jackdaw nest-boxes were put up in each of a number of experimental magpie territories. The nest-boxes were occupied immediately by jackdaws. Pairs of magpies served as controls and experimental birds in alternate seasons, so that their breeding success in the two situations could be compared. Combined data from all experimental and control pairs for the two seasons are shown in Table 1, and the reproductive output of individuals in experimental and control conditions is shown in Table 2: this comparison can be made only for birds that were individually marked and survived both breeding seasons. The start of laying and the size and weight of clutch were similar for the two groups, whereas experimental pairs produced fewer young, with lower weights at fledging, than did control pairs.

The competition coefficient for the effect of jackdaws on magpie reproduction (α_{mj}) is defined by:

$$N_{\mathrm{m}} = C - \alpha_{\mathrm{mj}} \times N_{\mathrm{j}} - D$$

where N_{m} and N_{j} are equilibrium numbers (adults plus nestlings) of magpies and jackdaws respectively, C is carrying capacity and D is the impact on magpie reproduction of competitors other than the jackdaw. Assuming identical C and D for experimental and control territories, the competition coefficient may be determined from

$$\alpha_{\mathrm{mj}} = \frac{N_{\mathrm{m}}\,(\text{control}) - N_{\mathrm{m}}\,(\text{exp.})}{N_{\mathrm{j}}\,(\text{exp.}) - N_{\mathrm{j}}\,(\text{control})}$$

This coefficient can be calculated on the following assumptions. (1) The difference in the numerator amounts to 1.35 magpie nestlings (Table 1). As Fig. 1 shows, the difference in reproductive output between pairs of experimental and control magpies is established well before the end of the nestling period. Thus, equilibrium seems to be attained. (2) The time spent by foraging adult magpies and jackdaws in 25 × 25-m plots was recorded 26–30 d after hatching, during 7 h of observation in each of three experimental and two control territories. Magpie foraging areas (≧ 1% of total magpie foraging time) were of similar size in experimental and control pairs, on the average 0.96 and 1.00 ha, respectively. The total number of 'jackdaw feeding minutes' in the magpie foraging areas of experimental pairs were 411, 563 and 575 and of control pairs 24 and 50. This corresponds to an average number of 1.23 foraging jackdaws in experimental magpie territories and 0.09 in control ones. As magpies foraged during 91% of total observation time, these figures are corrected to 1.35 and 0.10, respectively. Consequently, there was a mean difference of 1.25 foraging adult jackdaws between experimental and control magpie territories. (3) The mean number of nestlings in the jackdaw nest-boxes was 2.29 (s.e. = 0.19). Thus, 1.25 adult jackdaws provided food, both for themselves and for, on average, 1.25 × 0.5 × 2.29 = 1.43 nestlings. (4) As magpies and jackdaws hatch almost simultaneously and their nestlings weigh approximately the same, the energy requirements of the nestlings of the two species are almost identical. Thus, for the denominator in the above quotient to be directly comparable to the numerator (= 1.35 magpie nestlings) the denominator should be transformed into number of jackdaw nestlings. It is estimated that energy consumption of an adult jackdaw exceeds that of a nestling by about 35 to 40% (refs 3–4). Thus the difference between the numbers of jackdaws at experimental and control magpie territories, 1.25 and 1.43 nestlings, will be equivalent to a total of 3.15 jackdaw nestlings. As a result

$$\alpha_{\mathrm{mj}} = \frac{1.35}{3.15} = 0.43$$

This competition coefficient agrees fairly well with the predictions of niche overlap, based on circumstantial evidence.

A directional index[5] $[\alpha_{\mathrm{mj}} = (\sum_{\mathrm{h}} p_{\mathrm{jh}}\, p_{\mathrm{mh}})\ (\sum_{\mathrm{h}} p_{\mathrm{mh}}{}^{2})^{-1}]$ has often been used to estimate competition coefficients. Using this index and assuming equal extraction rates for all kinds of prey and equal consumption rates for the two competitors[6], values of 1.04 (based on bill dimensions, habitat distributions and feeding techniques) and 0.69 (based on food composition) are obtained for the competitive effect of jackdaw on magpie, and 0.59 and 0.71, respectively, for the reverse effect. This method leads to high expected competition coefficients, and it may not be so useful in natural conditions[7], as also indicated by the results reported here.

The competitive effects of jackdaws foraging in magpie territories were evident in the following sequence (Tables 1 and 2 and Fig. 1). The presence of jackdaws had no effect during the earlier stages of magpie breeding, before hatching. This is probably explained by a slight difference in time of laying, the magpie being 5 d ahead of the jackdaw, and the relatively small energy demands of the birds during the early phase of breeding. There was no sign of interspecific aggression during this period. After hatching, experimental magpies lost nestlings to a markedly higher degree than did control pairs. Both starvation and nest predation (chiefly by hooded crows, *Corvus cornix*) caused these losses, and the effects of these two factors were of similar magnitude. During this period magpies became highly aggressive—101 attacks on foraging jackdaws were recorded

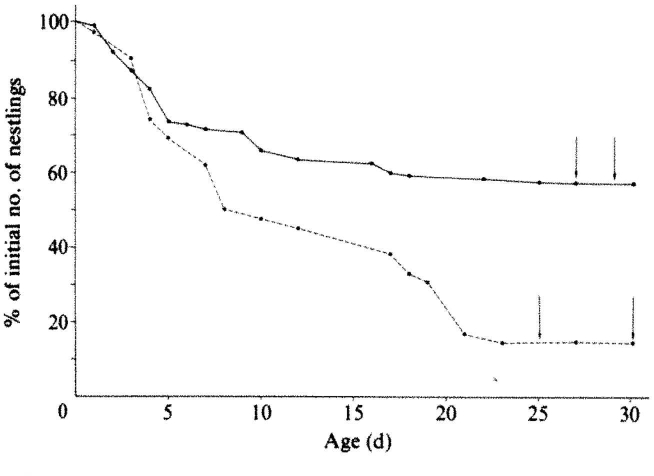

Fig. 1 Mortality of experimental (----, see text) and control (——) magpie broods. Arrows delimit periods of observation on jackdaw distribution in magpie feeding areas. In the control group there were 115 hatched young, and in the experimental group, 42.

Table 2 Breeding results of individual females in experimental and control conditions, in the same territory

Female	Start of laying (day 1 = 1 April) E	C	Clutch size E	C	Weight of clutch E	C	No. of fledged young E	C
1	17	8	6	7	45.0	50.4	0	3
2	20	23	6	6	60.0	61.2	2	3
3	25	29	5	6	52.5	63.0	0	3
4	26	28	7	7	68.6	70.0	1	1
5	13	11	7	7	77.0	79.1	0	0
6	21	16	6	6	64.2	62.4	0	0
7	20	28	8	7	—	—	0	4
8	—	—	—	—	—	—	0	0
9	—	—	5	6	—	—	0	4
P	NS		NS		NS		$t_8 = 2.78$ $P < 0.05$	

—, Data missing; NS, not significant.

during 35 h of observation. The jackdaws probably depleted available food in the magpie territories, thereby causing starvation of the magpie nestlings. Food depletion may have forced the parent magpies to forage farther from their nest than normal, thereby reducing the possibilities of defending their nest against predators. Furthermore, hungry nestlings beg for food loudly, thus increasing the risk of predator attraction.

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Plants metabolise ethylene to ethylene glycol

Dale C. Blomstrom & Elmo M. Beyer Jr

Central Research and Development Department, Experimental
Station, E.I. du Pont de Nemours and Company, Wilmington,
Delaware 19898

Ethylene is a natural plant growth regulator often produced in sufficient quantities to alter cellular and developmental processes in a characteristic hormonal manner[1]. Almost all phases of plant development are affected, including germination, growth, flowering, dormancy, abscission, senescence and sex expression. The earlier view that ethylene is metabolically inert[2–4] has been shown to be untenable[5–7]; low but readily detectable rates of ethylene metabolism have been observed in several plant tissues. These and other studies[8–10] indicate that this metabolism does not represent a system for the removal of the ethylene but instead represents the initial biochemical events in the action of ethylene in the plant. Here we report that one of the stable end-products of this ethylene metabolism is ethylene glycol, while for the ethylene mimic propylene, it is 1,2-propanediol.

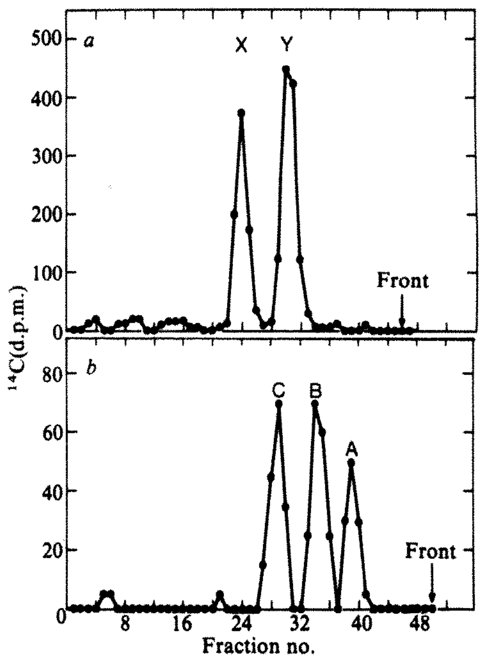

Fig. 1 Separation of neutral metabolites of *a*, ^{14}C-ethylene and *b*, ^{14}C-propylene by paper chromatography with butanol:acetic acid:water 3:3:2.

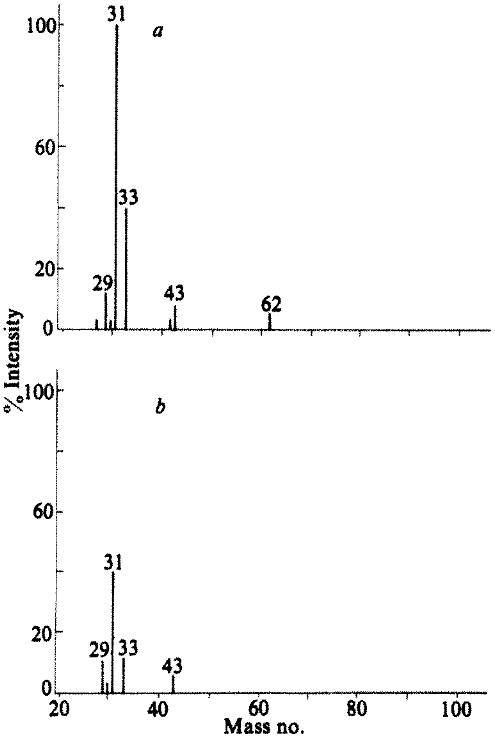

Fig. 2 Identification of ethylene metabolite Y (*b*) by mass spectroscopy. Mass spectra of metabolite isolated from ethylene-exposed pea tissue and of authentic ethylene glycol (*a*) were obtained by scanning effluent peaks from a Chromosorb 101 GC column, heated from 75 °C to 190 °C at 15 °C min^{-1}, with a Vacuum Generators 16F mass spectrometer. Because of the low concentration of metabolite in the extract its three weakest mass peaks were below the detection threshold and hence were absent from its spectrum.

Pea seedlings were surface sterilised as described previously[5] and germinated in the dark in sterile vermiculite. After 5 d the upper halves of the seedlings were removed, placed in glass stoppered flasks with 4 ml distilled water and exposed in the dark at 24 °C to 10 μl l^{-1} ^{14}C-labelled ethylene (Amersham,

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120 mCi mmol^{-1}) purified by preparative gas chromatography[11]. Incubation flasks contained a side-well with 1 ml 1 M NaOH to trap evolved CO_2. After exposure for 1 or 2 d, epicotyls were removed and a 50% ethanol extract of the tissue was prepared. After centrifugation the extract was freed of acids and bases by passage through columns of cationic and anionic exchange resins (AG50W-X8 and AG1-X8, Bio-Rad). Paper chromatography of the resulting neutral extract revealed two ^{14}C-labelled metabolites (Fig. 1*a*). The faster moving metabolite Y was investigated first because short-term labelling and pulse chase experiments indicated that it was formed before X. When compared to various neutral standard compounds, including oxidation products of ethylene, it always co-migrated with ethylene glycol (Table 1).

For confirmation by mass spectrometry, seedlings were grown in large trays for 5 d and exposed to 1,000 μl l^{-1} unlabelled ethylene for 2 d. Epicotyls were then collected and from 2,700 g fresh weight of tissue a neutral ethanol extract was prepared, spiked with ^{14}C-labelled metabolite Y, and purified for gas chromatography-mass spectrometry (GC-MS) using ion-exchange chromatography, acetonitrile precipitation and HPLC (silica column, mobile phase of butyl chloride : methanol : H_2O, 830 : 170 : 5). The mass spectrum of the Y peak emerging from a Chromosorb 101 GC column was of low intensity but essentially identical to that of authentic ethylene glycol (Fig. 2).

In similar studies with the ethylene mimic propylene, exposure of pea epicotyls to 10 μl l^{-1} of purified ^{14}C-labelled propylene (Amersham, 3 mCi mmol^{-1}) formed three ^{14}C-labelled metabolites (Fig. 1*b*). Co-chromatography with authentic material clearly showed that propylene metabolite A was 1,2-propanediol (Table 2), and GC-MS analysis of a purified sample from pea epicotyls provided positive identification (Fig. 3).

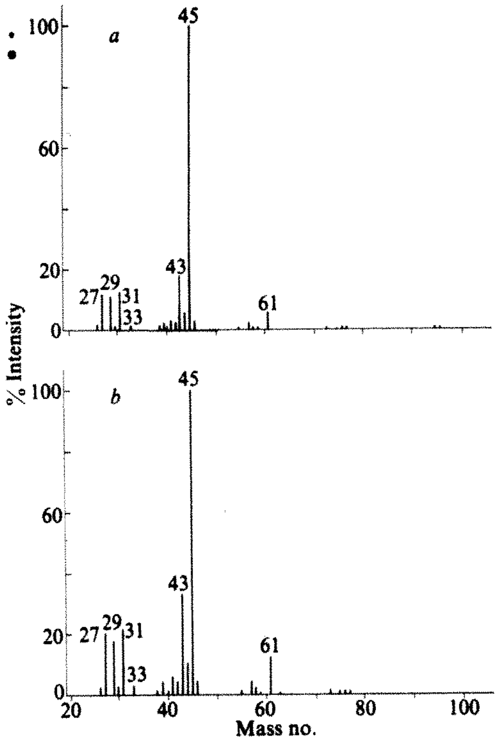

Fig. 3 Identification of propylene metabolite A (*b*) by mass spectroscopy. Mass spectra of purified metabolite from propylene-exposed pea tissue and of authentic 1,2-propanediol (*a*) were obtained as in Fig. 2.

Ethylene metabolite X and propylene metabolite C have been identified as glucose conjugates of ethylene glycol and 1,2-propanediol, respectively, and treatment of either with β-glucosidase yields the corresponding glycol. The formation of metabolites X and Y appears to be a general phenomenon associated with the physiological chemistry of ethylene in plants as we have detected them in a variety of tissues exposed to $^{14}C_2H_4$, including pea epicotyl tissue, carnation ovary tissue, morning glory flowers, cotton abscission zone segments and tomato fruit.

Table 1 Co-chromatography of ethylene metabolite Y and ethylene glycol

Chromatographic system	Solid phase	Mobile phase	Retention time or R_f value ^{14}C-ethylene metabolite Y	 Ethylene glycol
Liquid	Silica column	Acetonitrile : water (85 : 15)	6.50 min	6.41 min
Liquid	Silica column	Butyl chloride : methanol : water (75 : 25 : 0.5)	9.90 min	9.82 min
Liquid	Amine-bonded silica column	Acetonitrile : water (199 : 1)	3.70 min	3.70 min
Gas	Chromosorb 101 column, 200 °C	Helium	2.45 min	2.46 min
Thin layer	Cellulose	Ethyl acetate : acetic acid : formic acid : water (18 : 3 : 1 : 4)	0.72 (R_f)	0.72 (R_f)
Paper	Cellulose	Butanol : acetic acid : water (3 : 3 : 2)	0.64 (R_f)	0.65 (R_f)

^{14}C-ethylene metabolite Y was detected by scintillation counting in all systems. Ethylene glycol was detected by monitoring the refractive index in liquid systems, by flame ionisation in the gas system, and by scintillation counting of authentic ^{14}C-ethylene glycol in thin layer and paper systems.

Table 2 Co-chromatography of propylene metabolite A and 1,2-propanediol

Chromatographic system	Solid phase	Mobile phase	Retention time or R_f value ^{14}C-propylene metabolite A	 1,2-Propanediol
Liquid	Silica column	Butyl chloride : methanol : water (75 : 25 : 0.5)	7.59 min	7.65 min
Liquid	Silica column	Acetonitrile : water (98 : 2)	8.08 min	8.00 min
Liquid	Nitrile-bonded silica column	Butyl chloride : methanol : water (85 : 15 : 0.5)	7.87 min	7.85 min
Gas	Chromosorb 101 column, 200 °C	Helium	2.90 min	2.90 min
Thin layer	Silica	Butyl chloride : methanol : water (75 : 25 : 0.5)	0.50 (R_f)	0.48 (R_f)
Thin layer	Silica	Acetonitrile : water (95 : 5)	0.58 (R_f)	0.60 (R_f)

^{14}C-propylene metabolite A was detected by scintillation counting in all systems. 1,2-Propanediol was detected by monitoring the refractive index in liquid systems, by flame ionisation in the gas system, and by I_2 vapour staining in thin layer systems.

To our knowledge this is the first report demonstrating that higher plants oxidise ethylene to ethylene glycol. The importance of this oxidative process stems from its apparent direct involvement in the mechanism of action of this hormone[7]. It has been suggested[6] that this metabolism might occur at a Cu-containing receptor site, and this notion is supported by the sensitivity of this system to Cu chelators, COS and CS_2 (ref. 8). In nonbiological aqueous systems $Cu–C_2H_4$ complexes can undergo oxidation to yield ethylene oxide[12], and in *Vicia faba* L. cotyledons[13] ethylene was recently shown to be rapidly converted to this product. Although this rapid conversion seems to be unique to this tissue (ref. 14 and M. A. Hall, personal communication), it is of interest because ethylene oxide is a likely precursor of ethylene glycol. In conditions like those found in *Vicia faba* cotyledons, where ethylene is rapidly metabolised, perhaps ethylene oxide escapes before undergoing hydrolysis, or this latter step may be blocked in this tissue. Precedent for this can be found in microbial systems where epoxidation of ethylene apparently occurs but not glycol formation[15].

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Light-dependent synthesis of cholecalciferol in a green plant

Hermann Zucker, Heidi Stark & Walter A. Rambeck

Institute for Physiology, Physiological Chemistry and Nutrition Physiology, Veterinary Faculty, Ludwig-Maximilians-University, D-8000 Munich, FRG

Cholecalciferol (vitamin D_3) and several of its metabolites participate in the regulation of calcium and phosphorus metabolism in higher vertebrates (see refs 1, 2 for reviews). In contrast to the synthesis of ergocalciferol, the vitamin D of plant origin, cholecalciferol synthesis has been considered to be confined to skin lipids of vertebrates. Its 6,7-*cis*-intermediate, precalciferol[3], is formed by a photoreaction (wavelength 250–310 nm) from 7-dehydrocholesterol in or on skin tissue. Recently, however, a metabolite of cholecalciferol, 1,25-dihydrocholecalciferol, previously known to be synthesised only in mammalian and avian kidneys, has been detected in at least two species of Solanaceae[4,5]. Furthermore, Rambeck *et al.*[6] showed that cholecalciferol occurs in the common grass, *Trisetum flavescens*, which had been identified by Dirksen *et al.*[7] as the cause of calcinosis in grazing cattle. We now demonstrate that cholecalciferol is formed in *T. flavescens* only in the presence of UV light, thus suggesting a similar path of synthesis in plants to that which occurs in vertebrates.

For the present studies, *T. flavescens* and other grasses were sown in a dark room in wooden frames containing finely sieved soil and covered by cubic tents of aluminium foil to exclude all radiation from the outside. Inside, Grolux lamps of 30 W each were placed 60 cm above the soil surface. Spectral energy distribution as measured at soil level was: 780–400 nm, 97.5%; 400–315 nm, 2.1%; 315–280 nm, 0.4%. To exclude UV light entirely, yellow Plexiglass filters, which absorb wavelengths <490 nm, could be inserted horizontally beneath the lamps. Growth, as judged by appearance and dry matter yield per m^2, was not noticeably influenced by the absence of UV light. The grass was cut after 4–6 weeks and freeze dried taking care to prevent exposure to any source of light.

Table 1 Influence of *T. flavescens* grown with and without UV light on rat inorganic serum phosphorus (mg P per 100 ml)

	Test 1	Test 2	Test 3
Concentration of *T. flavescens* dry matter in test diet	6%	3%	2%
+UV grown	4.2±0.9	4.5±0.6	3.1±0.5
−UV grown	2.6±0.5	2.7±0.4	2.2±0.6
Negative control	1.4±0.1	2.5±1.1	1.7±0.4
Negative control + vitamin D_3	3.3±0.7	3.7±0.3	—

Weanling rats are placed on a vitamin D-free diet containing ~0.5% total P. Calcium concentration is ~0.4% for 14 days and is reduced to ~0.1% during the third week. The experimental diets, containing the respective vitamin D sources and ~4% Ca, are then given for 3 days.This change in Ca intake leads to a sudden drop in serum P from 9–12 to 1.5–2.5 mg per 100 ml. Orally administered vitamin D reduces this drop in P_i serum levels. P_i was measured 65 h after the diet switch. Six individually kept rats were used for each treatment.

The vitamin D activity of the samples was tested by the same procedure previously used for the purification and isolation of cholecalciferol from *T. flavescens*[6]. This test uses an acute, drastic drop in inorganic serum phosphorus level which is induced by dietary means in the rat and can be prevented by administration of vitamin D (see Table 1 legend). *T. flavescens* grown without UV light showed significantly less, but still some vitamin D activity compared with *T. flavescens* grown without the filters in three independent replications (Table 1). As we suspected that traces of light may have led to some vitamin D activity, in the following experiments, the final steps of preparation were carried out in total darkness. As an additional control, an equivalent amount of diethyl ether extract from *T. flavescens* grown without UV was tested after a short exposure to a UV lamp.

The results, shown in Table 2, indicate that vitamin D activity is completely dependent on UV exposure, and, in addition, that a single exposure of quite short duration, as was the case with the extract, results in considerable vitamin D activity. Therefore, UV radiation is unlikely to be a limiting factor of cholecalciferol synthesis in this plant even in natural growing conditions.

Further experiments, to be reported in detail elsewhere, showed that the vitamin D activity is mainly confined to the leaves[8]. The concentration of cholecalciferol as estimated by the quail eggshell test[9], rat test, GC and HPLC ranged from about 0.05 to 0.15 mg per kg of whole plant dry matter. Cholecalciferol concentration seems to be independent of the stage of vegetation, first or second cutting, soil, geographical area and altitude above sea level. Differences are due to the proportions of young leafy parts. Five different breeds of *T. flavescens* were of similar activity. However, samples of *Trisetum elatior* (*Avena elatior, Arrhenatherum elatius* L.), closely related to *T. flavescens*, were entirely inactive. A very small, insignificant elevation of inorganic serum phosphorus was obtained in our tests from

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Table 2 Influence of UV light on vitamin D activity of *T. flavescens* and *T. flavescens* extract as measured by inorganic serum phosphorus (mg P per 100 ml)

	Test 1	Test 2
Concentration of *T. flavescens* dry matter (or extract aliquot) in test diet	3%	3%
+UV grown	3.7 ± 0.6	—
−UV grown	2.0 ± 0.3	—
−UV grown extract UV exposed	—	4.0 ± 0.5
Negative control	2.4 ± 0.6	2.6 ± 0.6
Negative control + vitamin D_3	4.3 ± 0.7	3.5 ± 0.6

For methods see Table 1 legend. The extract from lyophilised *T. flavescens* (grown without UV) was prepared by diethyl ether extraction as described by Rambeck *et al.* and placed for 3 min under a laboratory UV lamp[6].

samples of *Dactylis glomerata*. This widely occurring grass had been reported earlier[10] to contain cholecalciferol, although the concentration was not mentioned. Cholecalciferol or its immediate precursors had not been detected in a study of sterol distribution in pasture grasses, which included *T. flavescens*[11]. This may have been due to the low concentration, which necessitates rather refined techniques for its detection[6].

Our results are the first demonstration of a UV light-dependent synthesis of cholecalciferol in an autotrophic plant. (We have previously shown[6] that the total vitamin D activity of *T. flavescens* can be attributed to cholecalciferol rather than to any of its metabolites.) Our findings may have some relevance to hitherto unresolved questions concerning the ultimate origin of cholecalciferol in the livers of marine fish. Holick *et al.*[12] recently reported that some marine fish are incapable of synthesising cholecalciferol in the absence of UV light and may not even metabolise cholecalciferol. Thus, cholecalciferol may be a rather inert constituent of the marine food chain, produced by as yet unidentified species of the autotrophic phytoplankton in the upper strata of the oceans, where some penetrating UV radiation may suffice for its synthesis in plant cells. The functions of 'vitamin D' in plant cells are unknown, but a stimulatory effect of high concentrations of vitamin D analogues, including cholecalciferol, on rhizogenesis was recently reported by Buchala and Schmid[13].

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A square bacterium

A. E. Walsby

Marine Science Laboratories, Menai Bridge, Gwynedd, UK

I have come across a bacterium which has the form of a thin square sheet. In most bacteria such a shape would be precluded by the osmotically-generated internal hydrostatic pressure but this organism, found in a saturated brine pool, has little or no cell turgor pressure. Its shape is probably determined by the pattern in which the cell envelope particles assemble. These square bacteria are so thin and transparent and are so unlike any bacteria previously described that I would have overlooked them if they had not possessed gas vacuoles, and I had not been looking for different forms of gas-vacuolate organisms. The squares derive buoyancy from their gas vacuoles and float at the brine surface.

Gas vacuoles are found exclusively in prokaryotic organisms and occur mainly in planktonic forms[1,2]. It has been proposed that, through providing or regulating buoyancy, they are important in positioning these organisms at particular depths in natural waters[2]. Surveys of freshwater lakes[3,4] suggest that gas-vacuolate bacteria are to be found in all lakes which are stable or stratified and I wondered whether this applied also to saline pools. Gas-vacuolate bacteria of the genus *Halobacterium* have been isolated from solar salt[5] and it has been speculated that their gas vacuoles buoy them to the surface of brine pools where the oxygen these obligate aerobes require is more readily available[6]. However, this has yet to be substantiated by field observations[2].

I collected brine from a salt crust forming at the surface of a hypersaline pool in a sabkra (a coastal plain at sea level) just south of Nabq, on the Sinai Peninsula. The chemistry of the pool and its location are described in detail by Krumbein *et al.*[7]. Microscopic examination revealed abundant gas-vacuolate organisms. At least five morphologically distinct forms were present, and of these the square bacteria were most abundant. Using a Hawksley standard counting chamber, I estimated that there were 7×10^7 squares per ml of liquid brine.

The presence of gas vacuoles in the various forms was demonstrated by comparing the appearance of individual cells before and after application of pressure (Fig. 1) as described previously[3]. Gas vacuoles are made up of minute hollow, proteinaceous structures called gas vesicles[8]. By virtue of their small size and the low refractive index of their contents, they scatter light very strongly and show as brilliant light objects against the dark ground of the cytoplasm under the phase contrast microscope. However, they can be made to collapse irreversibly by application of pressure and then they are no longer visible. This disappearance is diagnostic of gas vesicles—and the gas vacuoles they form—and identifies them unambiguously[2,3]. The presence of gas vacuoles in the squares firmly established their bacterial nature. Attempts to culture the organism were unsuccessful, but samples of brine were enriched for the square bacterium by centrifugation, as follows. A drop of the sample was mixed with the saline solution described below and placed in a haematocrit tube, forming a layer 18 mm deep. The tube was centrifuged at 227*g* for 100 min. The pressure generated at the base of the tube in these conditions was only 0.4 bars, too small to collapse a significant quantity of vesicles[9]. The buoyant, gas-vacuolate square bacteria rose to the meniscus from where they could be transferred by microsyringe. The squares also aggregated at the meniscus of the brine sample after leaving it to stand for two days. The squares which floated in contact with the coverslip of the counting chamber sank after application of pressures causing gas vacuole collapse. This showed that the square bacteria depended on their gas vacuoles for buoyancy.

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The membranous appearance of the squares at first suggested that they might be the walls of other disintegrated bacteria to which gas vacuoles had remained attached. However, some of the sheets, >20 μm^2, were clearly too large for this. Moreover, the thickness of the sheets, 0.2–0.5 μm, which could just be discerned from edge-on views, would be too much for a simple wall but just enough for a bacterium. This interpretation was supported by finding cells which were evidently in various stages of division (Fig. 2). The gas vacuoles were frequently concentrated around the edges of the squares and along the presumptive division lines. Undivided squares ranged in size from 1.5 to 11 μm (Fig. 2). Some squares had a number of division lines (strictly speaking, planes). Their arrangement indicated that the division occurred in two planes alternating at right angles; this produced sheets divided like postage stamps. Sheets of eight squares were not uncommon and sheets of 16 were found with individual squares tearing free.

Electron microscopy was carried out on samples of brine enriched for the square bacteria. The gas vesicles, which could be seen in whole-mounted unstained preparations, were similar to those of other bacteria, cylinders with cone-shaped ends[1,2]. Metal shadowing showed that the surface of the square bacteria was covered in particles ~2 nm across, arranged in a regular hexagonal lattice. This is similar to the arrangement observed in halobacteria[10] and suggests that, like them, the square bacteria belong to the group designated Archaebacteria[11]. Another feature of this group, satisfied by both, is that they occur in extreme environments. However, the phylogenetic relationships of the square bacterium can be established only by analysis of material from axenic cultures.

The regular array into which the cell envelope particles are assembled presumably determines the shape of the square bacterium. It is postulated that such a shape is permitted only by the absence of turgor pressure in this organism.

The majority of known bacteria have shapes such as spheres, ellipsoids or cylinders which are suitable for withstanding internal pressure. However, if pressure were generated in the square bacterium the flat surfaces would tend to bow outwards, break or come apart at the joining edges. Because the squares are obviously stable and remain flat they must have a low turgor pressure inside them. Another extreme halophile with gas vacuoles, *Halobacterium* sp.[12] has no detectible turgor pressure[13] and may, in certain conditions, produce rather flattened cells[10]. (*Simonsiella* spp. also have flattened cells, arranged in filaments[14], but nothing is known of their osmotic relationships.)

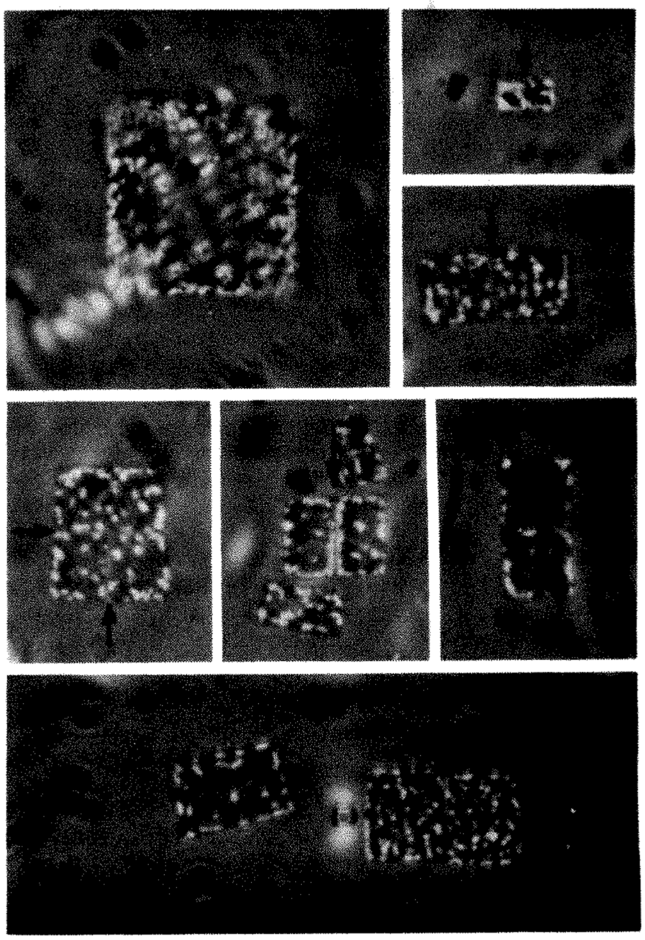

Fig. 2 Phase contrast light micrographs of the square bacterium illustrating the range of cell size. The light areas are gas vacuoles. Division lines, just detectable on the original print are indicated by the arrows. Scale bar, 10 μm.

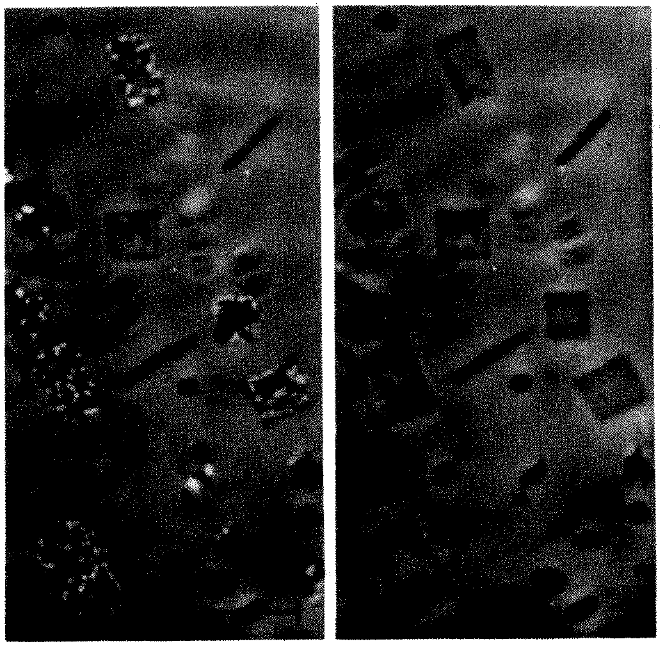

Fig. 1 Phase contrast light micrograph of a field of square bacteria seen before (on the left) and after (right) application of 5 bars pressure which causes the gas vacuoles to disappear. Scale bar 10 μm.

Cell turgor pressure can, in principle, be measured in any prokaryote which contains gas vacuoles. The constituent gas vesicles collapse irreversibly over a well-defined range of pressure. Inside the cell, turgor pressure contributes to the pressure required but if a non-penetrating solute, such as surcrose, is added to the suspending medium the turgor pressure is reduced and the pressure required to collapse the gas vesicles rises accordingly. Measurements on various freshwater prokaryotes suspended first in water and then in hypertonic sucrose solutions have revealed turgor pressures of mainly 2–5 bars[13], and increases in such pressures over this range cause the cells to swell[15]. The unusual absence of turgor in halobacteria is evidently related to the high salt concentration of their surroundings[13].

Accurate measurements of turgor pressure by the gas-vacuole method require small but homogeneous suspensions of the organism, though a rough estimate can be made from observations on the disappearance of gas vesicles in individual cells under the microscope. A 4-μl sample of the brine was mixed with 15 μl of 1 M sucrose dissolved in a salt solution containing NaCl (250 $g\,l^{-1}$), KCl (5 $g\,l^{-1}$), $MgSO_4$ (10 $g\,l^{-1}$) and $CaCl_2$ (0.13 $g\,l^{-1}$), the main mineral ingredients of a halobacterium culture medium[16]. A control sample was mixed with the same salt solution without sucrose. Drops of the mixtures were placed on Hawksley counting chambers, depth 0.02 mm, and a coverslip was positioned acentrically to provide an air gap to the channel surrounding the platform[3]. The bacteria were observed under phase contrast illumination using a ×1,000 oil immersion objective and again after pressurising the slide at 1, 1.5, 2, 2.5, 3 and 10 bars. Individual gas vesicles could be seen but not separately resolved at this magnification. In both mixtures with and without sucrose a definite reduction in gas vesicle numbers per cell was seen after application of 1.5 bars, most of the vesicles had disappeared by 2.5 bars, and none remained beyond 3 bars. The lack of difference between the two samples with respect to the pressure required to collapse gas vesicles shows that turgor pressure was small or non-existent[13].

In the absence of turgor pressure there is no obvious constraint on the shape of a bacterium and in theory any shape might be adopted, depending on the shape and arrangement of units which make up the cell wall. Whatever determines its shape apparently allows for some variation as occasionally triangular and trapezoid shapes are seen. These appear to be complete cells and not simply squares which have been folded or torn. The similarity in shape of the bacterium and the salt crystals with which it coexists is probably fortuitous.

The brine sample was collected on a visit kindly arranged by Professor Moshe Shilo and supported by the Royal Society. I thank Professor Wolfgang Krumbein for his geomicrobiological tour of Sinai and the brine pool, and Mr Ken Parkes for the electron microscopy.

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Cytoplasmic incompatibility in natural populations of a mosquito, *Culex pipiens* L.

A. Ralph Barr

Division of Epidemiology, School of Public Health,
University of California, Los Angeles, California 90024

When two strains of *Culex pipiens* (s.l.) of different geographical origin are cross-mated, the cross is frequently sterile in one or both directions[1]. Such incompatibility is said to be cytoplasmic because the crossability of a strain is determined by its maternal lineage[2]. The incompatibility is caused in some way by infection with a rickettsia-like bacterial symbiote, as removal of the symbiote abolishes the incompatibility[3]. Incompatibility has not been observed in crosses of American strains of *C. pipiens*[4–7]. On the other hand, most workers in other parts of the world who have crossed *C. pipiens* strains have noticed incompatibility[7–14], although there are no reports of incompatible egg rafts being collected in the field. We now report incompatibility in crosses of sympatric American strains of *C. pipiens* and the collection of incompatible egg rafts in the field.

On 4 July 1978, an egg raft of *Culex pipiens quinquefasciatus* Say was collected in Malibu, Los Angeles County, California. Its appearance suggested that the egg raft had been laid by a female which mated with an incompatible male; about 80% of the eggs showed evidence of embryonic development and few of these had developing setae visible, indicating that the embryos died early in development. About 20% of the eggs did not show evidence of embryonic development, and these were scattered throughout the raft rather than being in a group at one end. Three similar rafts have been collected from the same breeding place, on 15 July, 24 July and 22 August 1978. So far, 48 egg rafts have been examined from this breeding place, of which 43 hatched normally, 4 were apparently incompatible, and 1 showed no embryonic development at all, which indicates that it was probably laid by an uninseminated female.

Table 1 Crossing types of egg rafts collected in southern California

Locality	Crossing type* A	B	C	Total
Malibu	31	1	1	33
West Covina	8	1	0	9
West Los Angeles	7	0	1	8
Venice	3	0	0	3
Mar Vista	15	0	1	16
Total	64	2	3	69

* A, Reciprocally compatible with Californian crossing type; B, females incompatible with males of Californian crossing type, reciprocal cross compatible; C, males incompatible with females of Californian crossing type, reciprocal cross compatible.

After the second incompatible egg raft was observed (15 July), all egg rafts collected were taken to the laboratory, allowed to hatch, the larvae reared, and the adults reciprocally crossed with a mongrel *C. pipiens* laboratory strain of Californian crossing type (here designated crossing type A). Adults were placed in small cages (1.5-l capacity) and females fed on human or mouse blood. Thirty-three progenies have now been successfully tested; 31 were compatible when crossed in either direction with the laboratory strain. The females of one progeny (crossing type B) were incompatible when crossed with males of the laboratory strain, although the reciprocal cross was fertile. The males of another progeny (crossing type C) were incompatible with females of the laboratory strain, although the reciprocal cross was fertile.

In three other localities in greater Los Angeles (West Covina, West Los Angeles, Mar Vista), mixed populations of crossing types have also been found (Table 1), although incompatible egg rafts have not yet been taken in the field in these locations.

A laboratory strain was developed from sibling females of crossing type C which had been crossed with males of type A. In the F_4 of this strain, 10 of 60 egg rafts examined were incompatible. In the F_5, 2 of 39 examined were incompatible. This is the first report of incompatibility between individuals from a common female ancestor, although in this case the incompatible individuals may have been third or fourth cousins.

Variation in crossing type in the progeny of a female has been reported twice before[15,16], but incompatibility between individuals derived ultimately from a single female has not been reported. The present data suggest that there may be a mixed population of crossing type determinants in a female and these may segregate in her progeny to produce incompatibility in crosses between siblings. The cost of this incompatibility may be judged by the fact that 4 of 47 (8.5%) egg rafts of inseminated females taken at the Malibu breeding place failed to produce larvae.

It has been argued that two incompatible crossing types cannot exist for long in the same population because one type will eliminate the other in a fairly short time[17]. This argument is based on the assumption that each crossing type is invariable. It now seems probable that an individual mosquito may contain symbiotes producing two different and incompatible crossing types. A female may thus produce offspring which are of more than one crossing type and some offspring may be incompatible when mated with their siblings. Incompatibility in this case is a less effective selective agent for ridding the population of the incompatible crossing type. Incompatibility may thus persist in a natural population for a long time even though it is selected

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against when expressed. The consequence for the host population is reproductive waste and for the symbiotes a reduction in fitness of their hosts.

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Influence of H-2 complex on acquired resistance to *Leishmania donovani* infection in mice

Jenefer Blackwell, Joan Freeman & David Bradley

Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel Street, London, WC1

Innate susceptibility to *Leishmania donovani* infection in mice (measured over 2–4 weeks) is under the control of a single autosomal gene (*Lsh*) segregating for incompletely dominant resistant (*r*) and recessive susceptible (*s*) alleles[1]. This locus maps[2] away from the known histocompatibility loci to a position between the centromere and *Id-1* on the chromosome 1 of the mouse. Amongst homozygous recessive *Lsh*^{s} strains of mice two patterns of recovery are observed[3] when the course of infection is followed over a longer term. In some strains a dramatic fall in parasite numbers with histological liver damage occurs while other strains maintain immense parasite loads for up to two years involving mononuclear phagocytes throughout the body. We now present evidence which suggests that this difference in long-term response is largely controlled by a gene(s) within, or closely adjacent to, the major histocompatibility complex (H-2) of the mouse.

Initially C57BL/10ScSn and B10.D2/n congeneic mice plus their F_1, F_2 and backcross progeny were infected with *L. donovani* and the course of their infections followed over 130 days. In the parental strains all of the B10.D2/n mice maintained heavy infections over the 130 days while liver parasite burdens in C57BL/10ScSn mice began to fall by day 50. All of the F_1 and backcross to B10.D2/n mice followed the B10.D2/n pattern of long-term high infections. F_2 and backcross to C57BL/10ScSn mice appeared to be segregating for high and low parasite burdens by days 85 and 130. When graphed according to H-2 haplotype (Fig. 1), H-$2^{d/d}$ and H-$2^{b/d}$ mice always maintained long-term heavy infections while H-$2^{b/b}$ mice showed a reduction in parasite load. Although the time course for the early stage of this recovery differed slightly between experiments, a significant difference between H-$2^{b/b}$ and H-$2^{b/d}$ mice and between H-$2^{b/b}$ and H-$2^{d/d}$ mice was always observed 85 days after infection. H-$2^{b/d}$ and H-$2^{d/d}$ mice never differed significantly during the 130 days over which infections were followed. We concluded, therefore, that a gene(s) within, or closely adjacent to, H-2 controls an acquired resistance mechanism which behaves as a recessive trait and allows some innately susceptible mice to recover from an initially heavy infection of *L. donovani*. We propose *Rld-1* (recovery from *L. donovani*) as the provisional designation for this locus following the pattern laid down for H-2 linked genes controlling resistance to Gross leukaemia virus (*Rgv-1*)[6], Friend virus leukaemogenesis (*Rfv-1*)[7], and radiation leukaemia virus (*Rrv-1*)[8]. The two phenotypic patterns of long term infection we refer to as 'cure' and 'noncure'.

Following this discovery, four other H-2 haplotypes (*q*, *f*, *s* and *r*) on a B10 genetic background were tested for long-term response to *L. donovani* infection (Figs 2 and 3). The *q* (strain B10.G) mice showed the typical 'noncure' response, never differing significantly from control B10.D2/n and (B10.D2/n × C57BL/10ScSn)F_1 mice. The *f* (strain B10.M) mice also showed

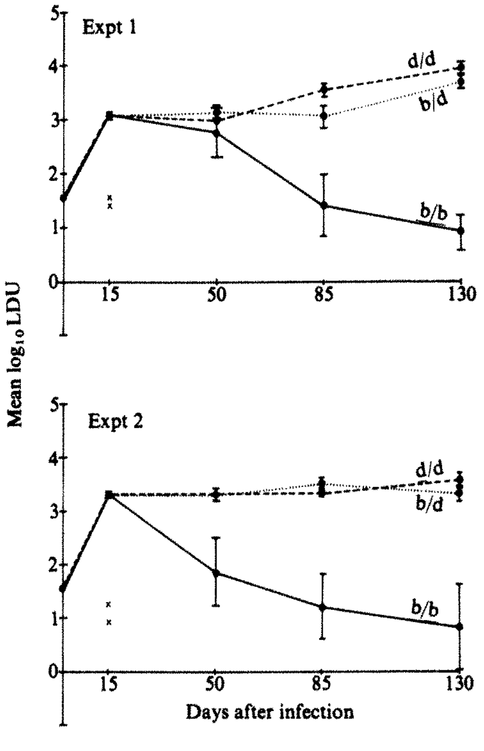

Fig. 1 For each experiment, groups of 10 mice from strains C57BL/10ScSn and B10.D2/n and from their F_1, F_2 and backcross progenies (London Hospital Immunology Department) were infected with 10^7 amastigotes *L. donovani* as described by Bradley and Kirkley[3]. Two mice from each group were killed on days 15, 50 and 85 following infection and four mice from each group on day 130. The number of parasites per 500 liver cell nuclei were counted from Giemsa-stained impression smears and the results expressed as 'Leishman Donovan units' (LDU)[4]. All mice except those killed on day 15 were individually H–2 haplotyped using microcytotoxicity testing[5] with two NIH private specificity antisera for each end (K and D) of H–2. Results graphed are mean $\log_{10}$LDU counts (±1s.e.) against days after infection for mice grouped according to the haplotypes: homozygous *b/b*, heterozygous *b/d*, and homozygous *d/d*. The day 0 points on the graphs represent approximately 70% uptake of the initial dose of amastigotes by the liver Kupffer cells found in many strains of mice by Bradley and Kirkley[3]. Since C57BL/10ScSn and B10.D2/n mice do not differ significantly in their day 15 parasite burdens[1], counts for all mice were pooled to obtain the day 15 points on the graphs. This is further justified by the very small standard errors (0.05 and 0.04) obtained for the 12 mice killed on day 15 in each experiment. Crosses (×) represent individual counts from control homozygous *Lsh*^{r} mice (C3H/He-*mg*/Ola and C57L) included in each experiment. Student's *t*-tests were used to test when differences in mean parasite loads between mice of different haplotypes became statistically significant. In both experiments *b/b* mice had significantly lower parasite loads than either *b/d* or *d/d* mice on days 85 ($P<0.02$) and 130 ($P<0.001$). In the second experiment the day 50 counts for *b/b* mice were already significantly lower ($P<0.02$). Mice of *b/d* and *d/d* haplotypes never differed significantly from each other.

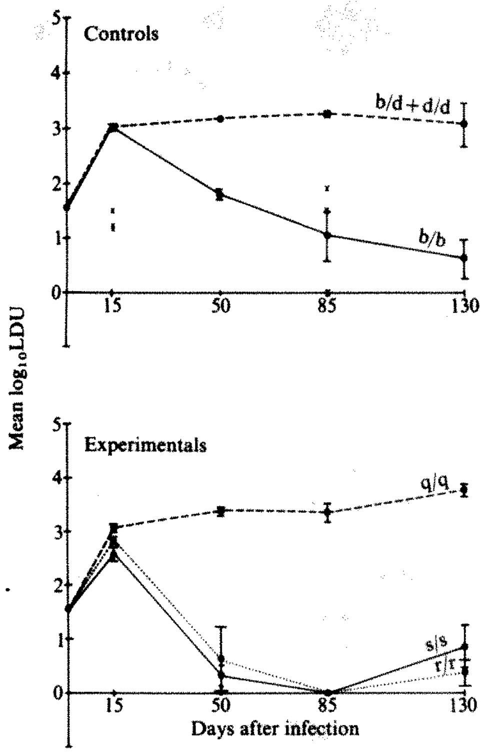

Fig. 2 Mice with *q*, *s* and *r* H–2 haplotypes on a B10 genetic background were tested for longterm response to *L. donovani* by infecting 17 B10.G/Ola, 15 B10.S/Ola and 15 B10.RIII(71NS)/Ola mice. For the B10.G strain, four mice were killed on each of days 15, 50 and 130 and five mice on day 85. For B10.S and B10.RIII strains, three mice were killed on day 15 and 4 mice on each of the days 50, 85 and 130. Control 'cure' and 'noncure' mice included 12 C57BL/10ScSn (Olac and Nuffield Foundation), 6(B10.D2/n × C57BL/10ScSn)F1 and 4 B10.D2/n/Ola mice. Since *d*/*d* (B10.D2/n) and *b*/*d* (F_1) mice do not differ significantly in their long-term response (Fig. 1), mice from these two groups were pooled as the 'noncure' control. Only three control mice were killed on the early days of infection allowing four 'cure' and four 'noncure' mice to be killed on each of days 85 and 130. Results graphed are mean $\log_{10}$LDU counts (±1 s.e.) for each group of mice against days after infection. Mean log counts for B10.S and B10.RIII mice did not differ significantly throughout the experiment. Neither did B10.G differ from control 'noncure' mice. B10.S and B10.RIII mice had significantly lower mean log counts on day 15 than both B10.G ($P<0.05$) and the control 'cure' and 'noncure' mice ($P<0.05$). Mean day 15 LDU counts for these two strains were still, however, 28-fold higher than mean LDU counts from homozygous Lsh^r mice (C3H/He-*mg*/Ola mice indicated by ×). Mean log counts also declined earlier in these two strains than in control 'cure' mice (day 50, $P<0.01$). This difference was no longer present on days 85 and 130. The levels of parasitaemia maintained in 'cure' mice at this stage in infection falls close to or below the threshold level for finding one parasite on an impression smear[9] and any slight differences between days and between strains are magnified by the log scale required to graph the extreme differences in parasite loads between 'cure' and 'noncure' mice. Hence we place no significance on the apparent increase in parasite loads between days 85 and 130 in B10.S and B10.RIII mice. These low levels of parasitaemia are of the same order of magnitude as those maintained in innately resistant (Lsh^r) mice (this experiment day 85 and Bradley and Kirkley[3]).

an essentially 'noncure' response. Although logLDU counts for these mice were slightly lower than control 'noncure' mice throughout the experiment, the difference only reached statistical significance ($P<0.01$) on day 85. Day 130 counts did not differ significantly between f haplotype and control 'noncure' mice. A significant difference was, however, observed between f haplotype and control 'cure' mice on days 85 ($P<0.01$) and 130 ($P<0.001$). The *s* (strain B10.S) and *r* (strain B10.RIII) haplotype mice displayed an acquired resistance mechanism similar to, but effective earlier than, that observed in C57BL/10ScSn *b* haplotype mice. Day 15 parasite levels in these two strains indicated that this acquired resistance mechanism may be operating sufficiently early to modify *Lsh* gene control of innate susceptibility. Parasite burdens were still, however, 28-fold higher than those observed in Lsh^r mice.

Our finding that 'cure' behaves as a recessive trait in H–$2^{b/d}$ mice is unusual in that most H–2 linked *Ir* genes show dominant responsiveness[10]. H–2 linked loci which do control apparent recessive immune responsiveness[7,11–13] do not follow the same distribution of haplotypes between 'responder' and 'nonresponder' strains as we have shown between 'cure' and 'noncure' strains. It is unlikely, therefore, that we are dealing with a known H–2 linked gene. Increasing evidence suggests that apparent recessive immune responsiveness may, in some cases, be due to dominant immune suppressor (*Is*) genes[14–16]. These *Is* genes exhibit many genetic similarities with *Ir* genes and map alongside them in the I region of the H–2 complex[15,16]. In other cases[7,13] apparent recessive immune responsiveness is controlled by genes mapping away from the I region of H–2 indicating a mechanism which may be quite independent of the *Ir* gene system[17]. If recovery from *L. donovani* infection were observed in other 'cure'/'noncure' heterozygotes, an immune suppression mechanism rather than recessive immune responsiveness would be favoured. We may thus be dealing with more than one locus within H–2, one controlling immune responsiveness and one controlling immune suppression. Varying rates of recovery between different 'cure' haplotypes could then be explained by a gene-dosage hypothesis. Our current examination of various heterozygous haplotypes as well as our attempts to map *Rld-1* within H–2 using recombinant haplotype strains should resolve these questions.

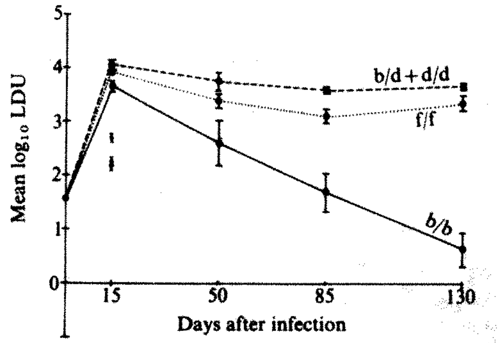

Fig. 3 Mice with the *f* H–2 haplotype on a B10 genetic background were tested for long-term response to *L. donovani* by infecting 15 B10.M/Ola mice. Three mice were killed on day 15, and four mice on each of days 50, 85 and 130. Three control 'cure' C57BL/10ScSn mice and three control 'noncure' (B10.D2/n × C57BL/10ScSn)F_1 mice were killed on each of days 15, 50 and 85, and four mice from each group on day 130. Three control 'noncure' B10.D2/n/Ola mice were also killed on day 85. Results graphed are mean $\log_{10}$LDU counts (±1 s.e.) for each group of mice against days after infection. Mean log counts for B10.M mice were slightly lower than control 'noncure' mice throughout the experiment but differed significantly only on day 85 ($P<0.01$). Control 'cure' mice had significantly lower mean log counts than both B10.M and control 'noncure' mice on days 85 ($P<0.01$, $P<0.001$) and 130 ($P<0.001$ in both cases). Crosses (×) represent individual counts from control homozygous Lsh^r (DBA/2) mice.

Finally, we have attempted to determine whether the acquired resistance mechanism operates for the same H–2 haplotypes on an alternative genetic background. Although chosen because BALB was the only other genetic background carrying the Lsh^s allele, BALB H–2 congenic strains did seem a particularly good choice for this experiment as BALB/c mice were already known to maintain chronic heavy infections involving mononuclear phagocytes throughout the body. BALB/c (*d* haplotype) and BALB/B (*b* haplotype) mice were tested for long-term response to *L. donovani* infection (Fig. 4). The acquired resistance mechanism of the *b* haplotype did appear to operate on the BALB genetic background.

Although marked H–2 restrictions have been demonstrated in the adoptive transfer of resistance to both bacteria[18] and viruses[19], the role of H–2 linked genes in determining actual resistance to infecting agents has previously only been defined for viruses. The present investigation was made possible only by our previous understanding of the genetic control of acute, or innate, immunity to *L. donovani* infection in mice, allowing this

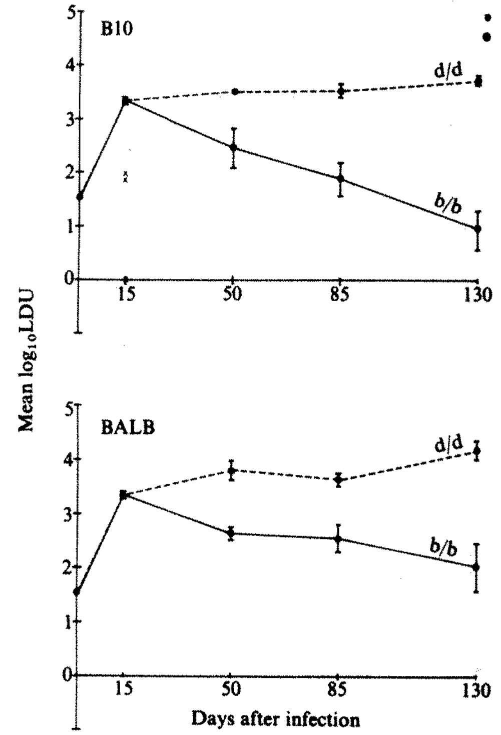

Fig. 4 Mice with *b* and *d* H-2 haplotypes on a BALB genetic background were tested for long-term response to *L. donovani* by infecting eight BALB/B/Ola and 10 BALB/c/Ola mice. For the BALB/B strain, two mice were killed on day 50 and three mice on each of days 85 and 130. For the BALB/c strain, one mouse was killed on day 15 and three mice on each of days 50, 85 and 130. Control 'cure' and 'noncure' mice included 15 C57BL/10ScSn (Olac and Nuffield Foundation) mice and 13 B10.D2/n (Olac and LSHTM) mice. Three mice from each control strain were killed on day 15, 2 on day 50, and five on day 85. Five C57BL/10ScSn and three B10.D2/n mice were killed on day 130. [The low number of BALB mice used in this experiment was due to a shortage in supply and this experiment is being repeated.] Although only one BALB/c mouse was tested here, BALB/c mice are known not to differ significantly in their day 15 mean log counts from C57BL/10ScSn and B10.D2/n mice[1]. We have tested this again many times (unpublished data) and have also subsequently tested the day 15 parasite levels in BALB/B mice. These do not differ significantly from BALB/c, C57BL/10ScSn or B10.D2/n mice. The day 15 points on the graphs therefore represent the mean log count (±1 s.e.) for the seven Lsh^s mice sacrificed on that day. Individual counts for homozygous Lsh^r (CBA/Ca/Ola) mice are indicated by a cross (×). Mean log counts obtained on days 50, 85 and 130 did not differ significantly between BALB/B and C57BL/10ScSn mice or between BALB/c and B10.D2/n mice. Mean log counts for BALB/B mice were significantly lower than BALB/c and B10.D2/n mice on days 50, 85 and 130 ($P<0.02$ in each case), as were C57BL/10ScSn mean log counts ($P<0.01$ in each case).

to be standardised in experiments analysing recovery and acquired immunity. Further study of the mechanisms involved in both the innate and acquired responses will be important to our understanding of this and other related human diseases, particularly in view of the recent implications of HLA linked susceptibility to leprosy[20,21] and malaria[22] in man. The mouse model allows a more precise correlation with major histocompatibility type, more scope for genetic manipulation, and a more direct access to the mechanisms involved.

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Formation of SV40 specific and H-2 restricted target cell antigen by somatic cell fusion

Terri S. Watt & Linda R. Gooding

Division of Immunology, Box 3010, Duke University Medical Center, Durham, North Carolina 27710

Cytotoxic T lymphocytes (CTLs) to syngeneic Simian virus-40 (SV40)-transformed target cells lyse only SV40-transformed cells which are identical to the killer cells at the K and/or D loci of the H-2 complex in mice[1,2]. In fact, CTLs have been shown to require autologous H-2 antigen expression for target cell lysis in a variety of syngeneic systems[3]. The dual specificity of these lymphocytes could indicate that both virus-coded and H-2 gene products are recognised and that interaction between viral and H-2 antigens on the tumour cell surface may be required to form the target antigen. However, serologically detectable SV40-specific antigens have not been found on the transformed cell surface. In addition, antisera to viral proteins do not block T-cell mediated lysis (CML) of syngeneic transformants[4]. If an SV40-specific moiety does not actually exist at the cell membrane, T-cell recognition might occur via an 'altered self' antigen where the virus codes for some activity which alters the H-2 antigens at the level of transcription or translation. To test this hypothesis the following experiments were designed: target cells were 'manufactured' by polyethylene glycol (PEG)-induced fusion of two cell lines; one being syngeneic to the CTL but not SV40 transformed and the other possessing the SV40 genome but differing from the effectors at the H-2 locus. The resulting multinucleated cells (heterokaryons) were then used as targets in the CML assay. Membrane components of heterokaryon cells have been shown to intermix[5]. In the present experiments we reasoned that if the target antigen is an H-2K or H-2D molecule which has been altered in transcription or translation, then simple mixing of membrane components would not lead to target antigen formation and cell lysis. If, on the other hand, separate SV40 induced and H-2 molecules are required for recognition then heterokaryon formation should lead to target antigen expression. Results described below support the latter hypothesis. Furthermore, these results show the simple mixing of cellular components is sufficient for a recognisable target antigen to form, and that protein synthesis is not required.

Cell lines which were chosen as parents for fusion were mixed together and plated on 100-m tissue culture dishes (Falcon). The density at which each cell line was used was determined by the growth rate of that line; slower growing cells were plated at larger numbers than fast growing cells (see Table 1). Cells were ready for fusion with PEG when light monolayers were

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Table 1 Fusion of cell lines of various antigen specificities

Target cells					% Specific lysis	
^{51}Cr labelled × unlabelled		Cell densities used for mixing	PEG	% Fusion efficiency	C3H anti-H-2^k/SV40	(H-2^d × H-2^b)F$_1$ anti-H-2^k
H-2^k/SV40	—	1.2 × 10^6	−	—	74%	59%
H-2^b/SV40	—	1.2 × 10^6	−	—	1%	−4%
H-2^d/SV40	—	1.2 × 10^6	−	—	10%	−6%
H-2^k	—	1.8 × 10^6	−	—	3%	73%
H-2^k/SV40*	—		−	—	42%	ND
H-2^k/SV40*	—		+	—	47%	ND
H-2^b/SV40*	—		−	—	2%	ND
H-2^b/SV40*	—		+	—	1%	ND
H-2^{k*}	—		−	—	5%	ND
H-2^{k*}	—		+	—	7%	ND
H-2^b/SV40	H-2^k/SV40		+	47%	42%	20%
H-2^d/SV40	H-2^k/SV40		+	45%	46%	34%
H-2^k/SV40	H-2^k		+	59%	37%	ND
H-2^k	H-2^b/SV40		+	43%	21%	21%

Cells were fused using the procedure of Davison and Gerald[6] with slight modifications. Parent cells for fusion were first labelled for 12 h with 100 μCi of $Na_2{}^{51}CrO_4$ (200–500 Ci g^{-1}, NEN, ^{51}Cr), then washed three times in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine and 1.0% antibiotic/antimycotic mixture (Gibco, DME 10), and collected using 0.02% trypsin. Tissue culture dishes (100 mm) were then inoculated with the two parent cell types at the cell densities given and incubated in DME 10 at 37 °C, 5% CO_2 and 95% air. After 12 h the medium was aspirated off and the dishes were washed one time with phosphate-buffered saline (PBS). The cells were then covered with 4 ml of fusing solution (47% polyethylene glycol 6000, Baker, 10% dimethyl sulphoxide, in modified Eagles medium). After 1 min at room temperature the fusing solution was thoroughly rinsed from the dishes with PBS. Dishes were then incubated at 37 °C in DME 10. After 2 h cells were removed with trypsin, washed once with modified Eagles medium containing fetal calf serum, glutamine and antibiotics as above (MEM 10) and an aliquot was removed to determine the efficiency of fusion. One drop of cell suspension was added to one drop of 0.1% acrydine orange-PBS solution and cells were immediately viewed under a Leitz fluorescence microscope. The per cent of the total nuclei present in multinucleated cells was determined and expressed as

$$\text{\% Efficiency of fusion} = \frac{\text{No. nuclei present in multinucleates}}{\text{Total no. of nuclei}} \times 100$$

The fused cell population was expected to contain parent cells, monokaryons and heterokaryons. To reduce the parent cell populations and to minimise homokaryon formation cells were subjected to PEG treatment in conditions which insured maximum fusion. In these conditions most multinulceated cells were found to contain more than 2 and up to 20 or more nuclei. The ^{51}Cr-release assay was performed in triplicate using effectors generated in a secondary *in vitro* sensitisation against H-2^k/SV40 cells as previously described[7] or against allogeneic spleen cells. At the end of the incubation period (6 h) aliquots were removed from assay wells and counted in Beckman Gamma 4000 counter. Results were calculated and are expressed as follows:

$$\text{\% Specific lysis} = \frac{E-C}{T} \times 100\text{, where}$$

E = ^{51}Cr released in the presence of sensitised spleen cells: C = ^{51}Cr released in the presence of non-sensitised spleen cells and T = total ^{51}Cr released by addition of 1 M HCl. Ratio of effector cells to target cells = 50 : 1. Cell lines are designated as follows: PSC3H (H-2^k/SV40), PSSW (H-2^b/SV40), SVBALB (H-2^d/SV40), MM5 (H-2^k). Mouse strains were C3H/HeJ and (B10.D2 × C3H.SW)F$_1$.

* These experiments were run separately and the per cent specific lysis is reported for an effector: target cell ratio of 15 : 1.

achieved. Cells were collected 2h after fusion and the efficiency of the fusion procedure was determined (for details see Table 1).

The efficiency of fusion was usually found to be between 50 and 80% and was probably dependent on the density and arrangement of cells on any particular tissue culture dish at the time of PEG treatment. After fusion with PEG, cells were prepared for incubation with CTLs to determine if target antigens were distinguishable on the heterokaryon cell surfaces. C3H/He effector T cells, specific for H-2^k/SV40 antigens, were elicited and a ^{51}Cr-release assay was performed as previously described[1] (Fig. 1). The results of the CML assay indicated that CTLs were able specifically to lyse at least some cells in the PEG-treated cell population. The parent cells themselves were not lysed by the effectors as they lacked either SV40 transformation or the H-2^k antigen. Furthermore, the per cent lysis of parent cells which had been subjected to the fusion procedure did not change significantly from the untreated controls. These data indicated that the heterokaryon cells had acquired the target antigen which neither parent by itself possessed and that PEG treatment alone did not cause binding and fusion of effector cells with 'incorrect' target cells.

Numerous reports have indicated that parental cells comprising heterokaryons are capable of inducing various activities in one another[8]. In fact, the SV40 T antigen has been shown to migrate in heterokaryons from the SV40-transformed nucleus to the nucleus of the normal parent[9]. Therefore, to confirm the hypothesis that target antigen formation involved simple mixing of two or more components already present in the transformed cell it was necessary to show that the SV40-transformed parent cells were not directing the non-SV40 cells to alter their 'self-antigens' at the levels of transcription or translation. It was predicted that if the target antigen on an SV40-transformed syngeneic cell was a product of freely moving moieties, then *de*

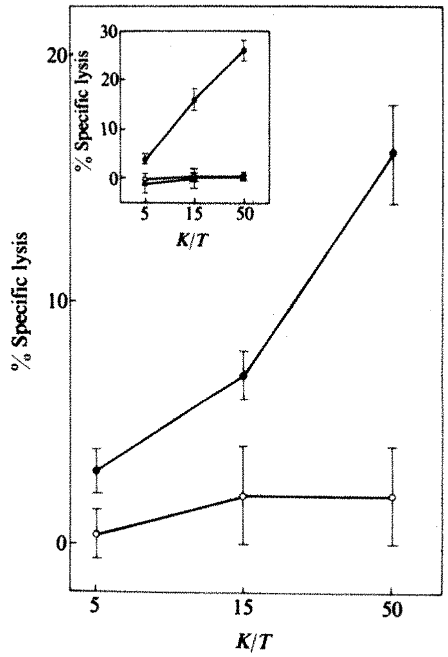

Fig. 1 CML of heterokaryon target cells by anti-H-2^k/SV40 restricted effectors at various killer to target ratios. Target cells are mixtures of H-2^k and H-2^b/SV40 cells fused with PEG (●), and H-2^K and H-2^b/SV40 cells not subjected to PEG treatment (○). All cells (including controls) were labelled at the time of mixing with 100 μCi ^{51}Cr per 100 mm dish. Cells were collected 2 h after PEG fusion with trypsin and used as targets in the ^{51}Cr-release CML assay (see Table 1). Insert: lysis of control targets by anti-H-2^k/SV40 effectors: H-2^k/SV40 (●): H-2^k (○); and H-2^b/SV40 (▲).

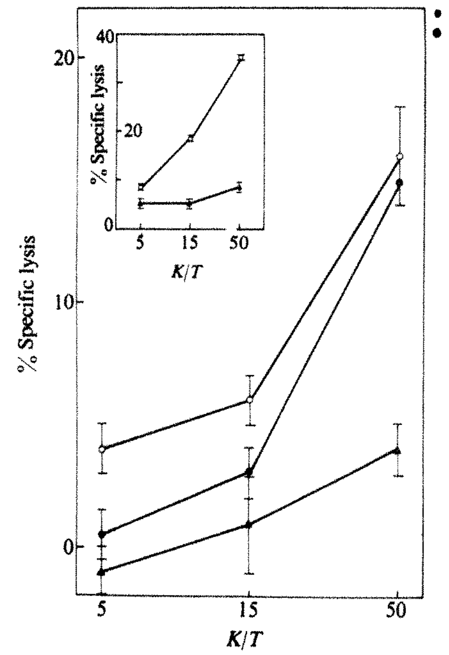

Fig. 2 Lack of a requirement for *de novo* protein synthesis in target antigen formation on heterokaryon target cells. Immediately after PEG fusion cells were incubated either in the absence (DME 10 − cyclo) or presence of 25 mM cycloheximide (DME 10 + cyclo). After 2 h cells were collected and aliquots removed for treatment with papain. Cells (2.5×10^6) in 0.5 ml were added to a solution containing 0.01 M cysteine, 50 μg DNase and 5 mg of papain. The volume was brought to 5 ml with DME 10 (with or without cycloheximide) and the tubes were incubated at 37 °C, 5% CO_2 and 95% air. After 60 min cells were centrifuged at 100*g* for 10 min, washed three times with DME 10 ± cyclo and prepared for use in the ^{51}Cr-release assay. H-2^k and H-2^b/SV40 cells were fused and treated with cycloheximide (●); papain (○); or cycloheximide and papain (▲). Effector cells in the CML assay were H-2^k/SV40 restricted. Insert: H-2^k/SV40 cells were treated with papain (○), or cycloheximide and papain (▲) along with the experimentals.

novo protein synthesis should not be required for target antigen formation in the heterokaryon cell. To address this point cycloheximide, an inhibitor of translation, was added to the incubation medium immediately after fusion with PEG (Fig. 2). The efficiency with which cycloheximide inhibited protein synthesis necessary for target antigen formation was tested by treating target cells with papain before the ^{51}Cr-release assay. Treatment with papain rendered the target cells refractile to lysis by the effectors probably by cleaving off part or all of the target antigen from the surface of the cell. Normally the SV40 target cell will synthesise more antigen and become susceptible to lysis by about 2 h (data not shown). Addition of cycloheximide effectively inhibited the reappearance of the target antigen following papain treatment both on the control targets (see insert Fig. 2) and on the heterokaryon cell population (Fig. 2). Although cycloheximide efficiently inhibited resynthesis of target antigens from the papain-treated cells, it had little effect on the 'manufacture' of target antigens on heterokaryons. Lysis of heterokaryon cell populations with or without cycloheximide by the anti-H-2^k/SV40 effectors was very similar.

These results are most consistent with the hypothesis that mixing of virus-induced and H-2 moieties is sufficient for target antigen formation in SV40-transformed cells. The data do not support any hypothesis in which the target antigen is produced before or during translation. Our results are similar to those seen by Sugamura *et al.* with T-cell mediated lysis of syngeneic Sendai virus-infected cells, where fusion of inactivated virus into the cell membrane was also sufficient to produce the target antigen[10].

Although these findings indicate that an 'altered-self' antigen is not produced by virus-directed transcriptional or translational modification of H-2 antigens, they do not rule out post-translational alterations. For example, the SV40-coded nuclear T antigen has been reported to have protein kinase activity[11], so that phosphorylation of H-2 molecules could produce the T-cell target antigen. Alternatively, T-cell recognition could require the formation of complexes between H-2 antigens and a virus-coded moiety on the cell surface. Finally, it remains possible that no interaction between virus and H-2 molecules is required, but that recognition is mediated via two separate receptors on the T cell for which only the proximity of molecules on the target cell surface is necessary.

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Chromosome abnormalities of leukaemic B lymphocytes in chronic lymphocytic leukaemia

John N. Hurley, Shu Man Fu & Henry G. Kunkel

The Rockefeller University, New York, New York 10021

R. S. K. Chaganti & James German

The New York Blood Center and Memorial Sloan-Kettering Cancer Center, New York, New York 10021

Chromosome analyses have revealed abnormalities in many cases of lymphoma, non-lymphocytic leukaemia and acute lymphoblastic leukaemia. Most studies on chronic lymphocytic leukaemia (CCL), however, have shown normal karyotypes[1–4] although chromosomal abnormalities have been reported[5,6]. In most of these studies specific surface marker analyses on leukaemic lymphocytes have not been carried out. It has been difficult to establish whether the metaphases seen in the majority of the CLL studies were from normal or from leukaemic cells. Recently, in one of our laboratories special interest has centred on cases of CLL with monoclonal serum protein bands; in two such cases with immunoglobulin M (IgM) bands, it was demonstrated with idiotypic antisera against the serum IgM band that the leukaemic lymphocytes were the precursors of plasma cells responsible for the monoclonal serum IgM[7,8]. In addition, the leukaemic lymphocytes of these patients could be stimulated *in vitro* to divide and to differentiate to plasma cells[8]. Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines were obtained from the two patients. With the idiotypic determinants as leukaemia specific markers, certain of these lines were shown to be derived from leukaemic cells[9]. Thus, rapidly proliferating populations of cells, which could be identified with certainty as being of CLL origin, were available for chromosome analysis. In the present study, conventional G- and Q-banding techniques for karyotypic analysis were carried out on the leukaemic lymphocytes induced to divide *in vitro* and on the cells of the leukaemic lymphoblastoid lines. Aneuploid karyotypes were found in the leukaemic B cells of both patients.

In the first case, Se, multiple cell lines were obtained[9] before the patient received chemotherapy or radiation. One of these lines, SeD, derived from splenic tissue, was shown to be derived

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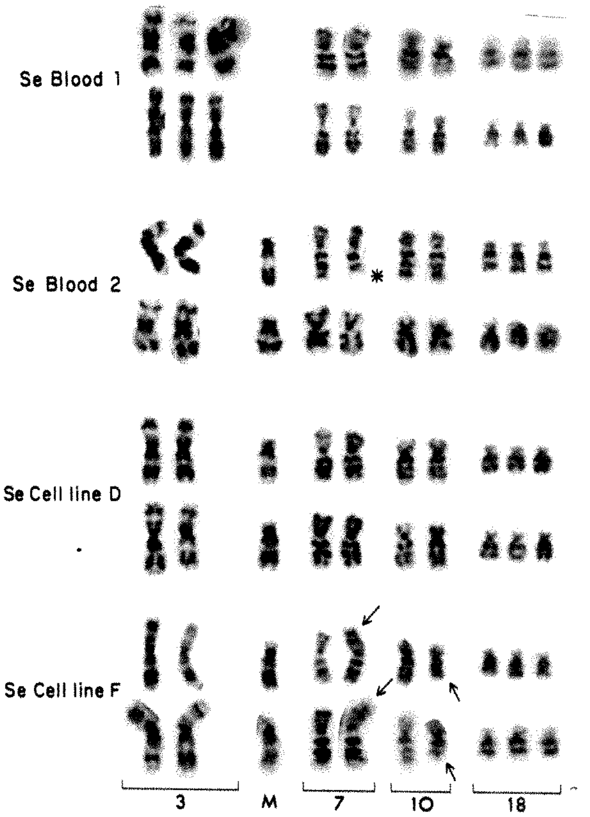

Fig. 1 Partial karyotypes showing all the abnormalities detected in the leukaemic cells from patient Se (G-banding). Cell line SeD, which had been derived from spleen before treatment, contains an extra chromosome 18 and an extra structurally abnormal chromosome (M) derived mainly from a chromosome 3. Cell line SeF, derived from peripheral blood after chemotherapy, contains both of the abnormalities found in cell line SeD plus an apparently balanced translocation between a no. 7 and a no. 10 (arrows). Leukaemic cells circulating in the blood (post-treatment) had two chromosome complements: one (Blood 2) was like that of cell line SeD which had been derived 30 months before the blood samples were taken, the other (Blood 1) differed from that of SeD only in having an extra intact no. 3 instead of the no. 3-derived marker chromosome (M). The terminal dark band missing from the long arm of one no. 7 chromosome of Blood 2 (*) was not found to be absent from any other cell; its significance is unknown.

from her leukaemic lymphocytes[9]. The modal number of chromosomes in line SeD was 48. This line's chromosome complement consisted of 46 apparently normal chromosomes, an extra no. 18 and an extra marker chromosome, M, which differs from any normal human chromosome as a result of structural rearrangement. The banding pattern of M suggests that it consists of the entire long arm, the centromere and a tiny segment of the short arm of a no. 3 to which a minute piece of some other chromosome has been translocated. The karyotype of cell line SeD can be described as 48, XX, +18, +M (Fig. 1). The extra chromosome 18 is apparently normal. Cell line SeF, a second leukaemic cell line, was derived from the patient's blood 2 yr after the establishment of line SeD and after chemotherapy with chlorambucil had been given. The leukaemic cell origin of this line was demonstrated by idiotypic analysis in a similar manner to that for SeD[9]. As in SeD, a modal chromosome number of 48 was observed in SeF, but an additional chromosome abnormality was present, a translocation between the long arms of a no. 7 and a no. 10. The karyotype of line SeF can be described as 48, XX, −7, −10, +18, +M, +t (7p; 10q) (Fig. 1). SeAl, one of the several lymphoblastoid cell lines from the same patient, derived from her non-leukaemic B cells[9], had a normal 46, XX karyotype.

In the previous studies[8], leukaemic B cells from patient Se matured to plasma cells *in vitro* in the presence of pokeweed mitogen (PWM) and autologous T cells. Of the plasma cells identifiable with the anti-idiotypic serum to the monoclonal IgM serum band (IgM Se), 15% showed ^{3}H-thymidine incorporation by autoradiography. The majority of the dividing cells in the non-T population stained with anti-idiotypic antiserum and thus were of leukaemic origin[8]. To obtain metaphases from dividing leukaemic B cells, Se peripheral blood lymphocytes were stimulated with PWM for 3 d, after which T cells were separated from non-T cells by the sheep erythrocyte rosette method and the chromosome complements in each fraction analysed. All the metaphases obtained in the T-cell fraction had a normal karyotype, 46, XX. However, in the non-T-cell preparation, a bimodal distribution of chromosome numbers was observed, with the major mode having 48 chromosomes and the minor 46 chromosomes (Table 1). Of 50 G-banded cells, 24 had the complement 48, XX, +3, +18 and 5 had 48, XX, +18, +M (Fig. 1). A substantial number of the metaphases had a normal karyotype, which was probably from contaminating dividing normal cells. In addition, a normal karyotype was found in metaphases obtained directly from Se's freshly aspirated bone marrow cells as well as her peripheral blood lymphocytes stimulated with phytohaemagglutinin (PHA).

The chromosome abnormalities observed in the cell lines and the *in vitro* induced dividing peripheral blood leukaemic lymphocytes from patient Se are summarised in Fig. 1. Trisomy 3 and trisomy 18 seem to represent the earliest detected alterations in the patient's leukaemic clone. As the karyotype of the clone evolved, M replaced a chromosome 3 and, later, translocation between chromosomes 7 and 10 occurred. In chronic myelogenous leukaemia entering the blastic phase, sequential alterations of the karyotype of this type have been found to be superimposed on the Ph^1 translocation[10]. In CLL patient Se, correlation between the observed sequential alteration and altered clinical activity was not apparent.

Similar cytological studies were carried out on selected cell lines[9] derived from the peripheral blood of CLL patient Ei after she had been treated with chlorambucil and prednisone. An idiotypic antiserum against the IgM Ei serum protein demonstrated that cell lines Ei 25, Ei 28 and Ei 81 were derived from her leukaemic lymphocytes[9]. All three leukaemic lines analysed had a modal chromosome number of 47, with the karyotype 47, XX, +12, and were, therefore, trisomic for what seems to be a normal chromosome 12. The lymphoblastoid lines Ei 151 and Ei 152 were shown by idiotypic analysis to be derived from non-leukaemic B cells, and the karyotypes of these two non-leukaemic lines were normal (46, XX). The finding of trisomy 12 in all three independently derived leukaemic cell lines and its absence in the two normal lines suggest that the chromosome abnormality is not a result of changes produced by chemotherapy or EBV transformation and cell culture.

Simultaneous trisomy of chromosomes 3 and 18 has been described in the non-Hodgkin lymphomas[5], although a structural rearrangement of chromosome 14 (14q+) is the most frequent abnormality[1,2,5]. A 14q+ chromosome has also been found in two patients with chronic T-cell leukaemia[6], in two CLL patients with undetermined cell types[5] and in a patient with a B-cell CLL[11]. Trisomy 12 has been observed in at least two patients with hairy cell leukaemia[12]. In the present studies, the finding of trisomies 3 and 18 in patient Se and trisomy 12 in patient Ei suggest similar chromosome abnormalities in cases of CLL, malignant lymphoma and hairy cell leukaemia. None of

Table 1 Chromosome numbers in pokeweed mitogen-stimulated peripheral blood mononuclear cells from patient Se after depletion of T cells

	Chromosome nos per cell						No. of cells examined
	≤44	45	46	47	48	49	
G-banding	1	6	11	2	29	1	50
Q-banding	0	0	6	4	20	0	30
Total	1	6	17	6	49	1	80

these chromosome abnormalities is known to be associated specifically with a particular type of lymphoproliferative disorder.

Thus, the leukaemic cells of both patients with CLL analysed in this study showed chromosome abnormalities. These were demonstrated in the *in vitro* stimulated cells from patient Se and the virus-transformed cell lines derived from both patients. The finding of normal karyotypes in the metaphases obtained from fresh bone marrow cells, PHA-stimulated cells and PWM-stimulated T cells in patient Se emphasises the difficulties inherent in cytogenetic studies of CLL. Without the idiotypic determinants as specific markers of the leukaemic cells, it would have been difficult to identify the leukaemic cells and demonstrate the associated chromosome abnormalities. The trisomy 3 and 18 in one patient and the trisomy 12 in the other are among the first well documented karyotypic abnormalities in chronic lymphocytic leukaemia of the common B-cell type. The fact that both patients studied showed abnormalities suggests that they occur at high frequency in cases of CLL but the exact incidence can be determined only when methods for identifying and inducing division of the leukaemic cells have been developed.

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Inability of the C3a anaphylatoxin to promote cellular lysis

Michael G. Goodman & William O. Weigle

Department of Immunopathology, Scripps Clinic and Research Foundation

Tony E. Hugli

Department of Molecular Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

Investigation of the biological effects of the cleavage product, C3a, derived from the third component of complement has generally focused on the effects it exerts as an anaphylatoxin[1], that is, smooth muscle contraction[2] and histamine release from mast cells[3,4] and basophils[5,6]. Therefore, reports that C3a exerts cytoxic effects on various cell types [7–9], and exhibits concentration-dependent specificity for malignant cells[9], excited great interest. The cytolytic activity mediated by macrophages which were activated *in vitro* may be attributed to their ability to generate C3a[10]. The far-reaching implications of such a cytolytic role for C3a led us to pursue these observations further. We report here that we have been unable to duplicate the results reported by Ferluga *et al.*[9]. On the contrary, we find that C3a actually reduces spontaneous release of label below normal background levels.

Human, porcine and rat C3a were purified according to established procedures[11,12]. Purity of the final products was assessed by polyacrylamide gel (*p*H 4.5) and cellulose acetate strip (*p*H 8.5) electrophoresis. All preparations were judged homogeneous based on the criteria of electrophoretic behaviour and a distinct amino acid composition. Functional activities of the anaphylatoxins were determined by the guinea pig ileal assay and from the ability to enhance vascular permeability in guinea pig skin[1]. Human $C3a_{desArg}$ was prepared according to the procedure of Hugli *et al.*[11] except that the serum carboxypeptidase B inhibitor, 6-aminohexanoic acid, was omitted during the complement activation step.

Table 1 Effect of human C3a on murine target cells

Expt 1

C3a concentration ($\mu g\ ml^{-1}$)	P-815 Cells ^{51}Cr release (c.p.m. per culture)	P-815 Cells % Specific release
0	190 ± 29	0
1	159 ± 19	−5
3	152 ± 22	−6
10	174 ± 5	−2
30	167 ± 3	−3
100	162 ± 18	−4

Expt 2

C3a concentration ($\mu g\ ml^{-1}$)	P-815 cells ^{51}Cr release (c.p.m. per culture)	P-815 cells % Specific release	Spleen cells ^{51}Cr release (c.p.m. per culture)	Spleen cells % Specific release
0	768 ± 46	0	1,494 ± 59	0
1	752 ± 86	−1	1,203 ± 101	−30
3	738 ± 41	−1	1,112 ± 123	−39
10	715 ± 19	−2	1,051 ± 54	−45
30	661 ± 57	−5	1,007 ± 87	−49
100	636 ± 17	−6	727 ± 29	−78

In expt 1 a total of 5×10^6 P-815 cells were labelled with ^{51}Cr-labelled sodium chromate in 1 ml medium and incubated at 37 °C for 1 h. Total release was 868 ± 0 c.p.m. ^{51}Cr release was assayed after 6 h of incubation. Each culture contained 3.5×10^4 target cells + the concentration of C3a indicated. In expt 2, 5×10^6 P-815 or spleen cells were labelled with ^{51}Cr-labelled sodium chromate in 0.3 ml medium and incubated at 37 °C for 1 h. Total release was 3,131 ± 89 c.p.m. for P-815 cells and 2,480 ± 57 c.p.m. for spleen cells. The per cent specific release of label from target cells attributable to the action of C3a was calculated according to the formula:

$$\frac{(\text{Experimental release} - \text{spontaneous release})}{(\text{Total release} - \text{spontaneous release})} \times 100$$

Total release was evaluated by adding a drop of 10% SDS to cultures containing target cells only. Data are presented as the arithmetic mean of triplicate cultures ± s.e.

Dissociated suspensions of murine spleen cells were prepared and cultured in RPMI-1640 based medium as detailed elsewhere[13]. This medium was supplemented with bovine serum albumin (BSA) or heat-inactivated fetal calf serum (FCS) as indicated. FCS was tested for and found deficient in carboxypeptidase B activity. The $H\text{-}2^d$ mastocytoma P-815 and the $H\text{-}2^b$ lymphoma EL-4 were maintained *in vivo* in DBA/2 and C57BL/6J mice, respectively. Target cells were labelled according to several different protocols. For the first protocol, 5×10^6 viable target cells were suspended in 1 ml of medium together with 100 μCi of ^{51}Cr-sodium chromate, and incubated for 1 h at 37 °C. Target cells were washed four times and resuspended at a density of 3.5×10^5 per ml in medium containing 0.1% crystallised BSA. In other experiments the same labelling protocol was followed except that the total volume of incubate was 0.3 ml. The use of this latter protocol resulted in a higher degree of labelling per cell. Several other labelling techniques were tested to optimise the amount of label taken up per

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Table 2 Effect of C3a from different species on P-815 target cells

	Human C3a		Rat C3a		Porcine C3a		Human C3a$_{\text{des Arg}}$	
C3a (μg ml^{-1})	^{3}H-TdR release (c.p.m. per culture)	% Specific release	^{3}H-TdR release (c.p.m. per culture)	% Specific release	^{3}H-TdR release (c.p.m. per culture)	% Specific release	^{3}H-TdR release (c.p.m. per culture)	% Specific release
0	6,513 ± 375	0	6,513 ± 375	0	6,513 ± 375	0	380 ± 100	0
1	4,773 ± 418	−3.3	5,987 ± 431	−1.0	4,998 ± 173	−2.9	335 ± 34	−0.2
3	4,091 ± 211	−4.6	6,024 ± 190	−0.9	3,861 ± 135	−5.0	345 ± 15	−0.2
10	3,568 ± 108	−5.6	5,373 ± 331	−2.0	4,974 ± 287	−2.9	298 ± 11	−0.4
30	2,592 ± 27	−7.4	4,604 ± 364	−3.6	4,005 ± 109	−4.7	269 ± 26	−0.5
100	2,820 ± 42	−7.0	3,469 ± 188	−5.8	3,610 ± 238	−5.5	231 ± 13	−0.7

^{3}H-TdR release was assayed after 6 h of incubation. Each culture contained 5×10^{4} target cells plus the concentration of C3a indicated. Total release was 59,427 ± 4,386 c.p.m. per culture. Data are presented as the arithmetic mean of five replicate cultures ± standard error. For the human C3a$_{\text{des Arg}}$ experiment ^{3}H-TdR release was also assayed after 6 h of incubation and each culture contained 5×10^{4} target cells. The concentration of C3a$_{\text{des Arg}}$ is indicated and total release was 23,010 ± 530 c.p.m. per culture. Release in the cultures with 100 μg ml^{-1} of intact C3a instead of C3a$_{\text{des Arg}}$ was 195 ± 12 c.p.m. per culture (−0.8% specific release).

cell. The most satisfactory protocol on this basis, was that described by Bloom *et al*[14]. Briefly, target cells were incubated in the same RPMI-1640 based medium used previously with the exception that BSA was omitted and 10% heat-inactivated FCS as well as 5×10^{-5}M 2-mercaptoethanol were added. 10^{7} target cells were incubated in 60-mm Petri dishes with 5 μCi ml^{-1} of ^{3}H-TdR in a volume of 10 ml. Cultures were incubated for 18–24 h, washed four times, resuspended to 10^{6} cells per ml in serum-free medium and used directly.

^{51}Cr-labelled target cells were assayed according to the procedure of Ferluga *et al.*[9]. Briefly, 3.5×10^{4} target cells in a volume of 0.1 ml medium were combined with various concentrations of C3a (in 20 μl of gelatin–saline) in 12 × 75-mm round bottomed tubes and incubated in an atmosphere of 5% CO_2 at 37 °C for the time indicated. At that point, 1 ml of 5% FCS-containing medium was added per tube, samples were mixed gently, centrifuged, and 0.5 ml of the supernatant was removed and counted in a Searle 1195 automatic gamma counter. Target cells labelled with ^{3}H-TdR were assayed as follows: 5×10^{4} target cells in a volume of 50 μl were added to microculture wells (Falcon Plastics no. 3040, 3041) together with a 2× concentration of human C3a in a volume of 50 μl of gelatin–saline. Cells were incubated for 6 h unless otherwise stated, at which point the assay was terminated by adding 100 μl of medium containing 10% FCS to each well. The microculture plate was then centrifuged and 100 μl per culture was harvested into scintillation vials containing 3 ml of Aquasol. Samples were counted in a Beckman LS-230 liquid scintillation counter.

The data presented in Table 1 are results from experiments in which variable concentrations of human C3a were incubated with two different types of radiolabelled target cells. Significant release of label from the target cells, an event that would indicate lysis initiated by C3a, failed to occur. Although these experiments were performed repeatedly and the methodology specified by Ferluga *et al.*[9] was adhered to fastidiously, lysis was never observed. This was true of ^{51}Cr-labelled P-815 target cells, the cell type reported to be the most readily lysed, as well as EL-4 and normal murine splenic lymphocytes. The susceptibility of these target cells to lysis, as judged by their release of the ^{51}Cr label, was previously established by incubating the cells with allogeneic cytotoxic T lymphocytes which had been specifically educated *in vivo*. The range of C3a concentrations tested was precisely that which has been previously reported to produce lytic activity against labelled target cells[9]. The assays presented in Table 1 were performed after 6 h of incubation, as specified by Ferluga *et al.*[9]. However, in other experiments (data not shown) the kinetic profile (between 2 and 24 h of culture) was examined. The results of these experiments demonstrated that lysis could not be induced regardless of the duration of the incubation. Viability studies showed no differences in cell number or in per cent viability between control and C3a-incubated cultures (data not shown).

As our next approach, highly purified C3a preparations of human, rat, and porcine origin were studied to determine whether the inability of human C3a preparations to lyse target tumour cells might be simply a species limitation. The results of experiments using ^{3}H-TdR-labelled P-815 mastocytoma target cells are presented in Table 2. Here again, the actual counts released per culture, as well as the per cent specific release, are presented as a function of the concentration of C3a used in culture. Once more it is apparent that there is no lysis induced by C3a in excess of the background spontaneous release regardless of its species derivation, over the entire range of concentrations examined in Table 1. Although guinea pig C3a was used in the studies of Ferluga *et al.*[9], the use of C3a from a variety of species (Table 2) makes it unlikely that cytolysis is peculiar to the guinea pig molecule especially since the target cells were xenogeneic. Moreover, it appears that if any activity can be ascribed to the presence of C3a in culture, that activity would be a diminution of the spontaneous release of radioactive label from target cells. The fact that biology inactive C3a$_{\text{des Arg}}$ exhibits the same effect as intact C3a on spontaneous release argues that this phenomenon is not an anaphylatoxin-specific effect.

In other studies, the effect of C3a on isologous target cells was studied. Human lymphoblasts, generated from PBL (activated by staphylococcal protein A under optimal mitogenic conditions for 6 d) were incubated with concentrations of human C3a

Table 3 Effect of polyamines on spontaneous lysis of murine tumour cells

	Human C3a		Spermidine	
Concentration (μg ml^{-1})	^{3}H-TdR release (c.p.m. per culture)	% Specific release	^{51}Cr release (c.p.m. per culture)	% Specific release
0	5,826 ± 440	0	5,826 ± 440	0
1	3,524 ± 90	−4.5	3,599 ± 173	−4.3
3	3,702 ± 124	−4.1	2,829 ± 223	−5.8
10	3,300 ± 34	−4.9	1,735 ± 128	−7.9
100	3,143 ± 97	−5.2	589 ± 34	−10.2

^{3}H-TdR release was assayed after 6 h of incubation. Each culture contained 5×10^{4} P-815 target cells plus the concentration of C3a or spermidine indicated. Total release was 57,328 ± 5,553 c.p.m. per culture in these experiments.

ranging from 1 to 200 μg ml^{-1}. The results of these experiments demonstrated that purified human C3a was incapable of mediating the release of label from human lymphoblastoid target cells.

Theoretically C3a should be able to interact with cells either by high or low affinity receptor–ligand binding, or by low affinity nonspecific interactions (such as salt bridging and hydrogen bonding). In a recent paper, Glovsky and colleagues[6] studied the binding of purified human C3a to human eosinophils, neutrophils, basophils, monocytes and lymphocytes. Of particular interest was their observation that virtually no detectable binding of human C3a to peripheral blood lymphocytes occurred. In the light of these findings, it is difficult to comprehend the rational basis for specific lysis of human lymphoblasts by C3a observed by Ferluga and coworkers[9]. One would have to postulate a nonspecific or low affinity mechanism to account for such findings since high affinity binding of C3a to a specific cellular receptor is apparently ruled out.

On the contrary, diminution of spontaneous cytolysis by C3a was conceptualised as a nonspecific effect of polycations. To evaluate this possibility salmon spermidine, a polyamine of comparable size to C3a, was evaluated for its influence on the spontaneous lysis of labelled target cells. Table 3 demonstrates that increasing the concentration of salmon spermidine does in fact produce a diminution in the spontaneous release of cellular label. The effect of spermidine in our case is not likely to be due to inhibitory metabolites produced by polyamine oxidase contained in FCS acting on spermidine as a substrate[15]. It has been demonstrated that inhibitory effects attributable to such metabolites are reversed by washing, and in the present studies cells were washed four times before addition of spermidine in serum-free medium. Moreover, the same effects of polycations were observed when target cells were labelled in the absence of serum. The decrease in spontaneous release is interpreted as indicating that polyamines, similar in size and charge to C3a, are capable of exerting either direct or indirect nonspecific stabilising effects on the cell. This same conclusion has been independently corroborated by experiments in which human C3a was incubated with human peripheral blood neutrophils. Under those circumstances, the spontaneous release of lactate dehydrogenase (LDH) decreased concordantly with increasing concentrations of C3a (D. E. Chenoweth, personal communication).

We thus find no evidence to support the contention that C3a plays a significant cytolytic role in the host defense mechanisms against neoplasia.

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Prostaglandin synthesis in different phases of phagocytosis in lung macrophages

Wei Hsueh, F. Gonzalez-Crussi & Eric Hanneman

Department of Pathology of The Children's Memorial Hospital, Northwestern University Medical School, Chicago, Illinois 60614

Macrophages have a central role in bodily defence and inflammatory responses. Prostaglandins (PGs), mediators of inflammation[1], are secreted by macrophages during phagocytosis. PGE-like activity was first demonstrated in macrophage-rich peritoneal exudate cell preparations from guinea pigs[2,3]; later, other kinds of PGs were also found to be released by mouse peritoneal macrophages in response to inflammatory stimuli[4–6]. We have shown that rabbit alveolar macrophages also produce various PGs in response to phagocytic stimuli like zymosan and heat-killed bacteria[7,8]; however, it was not known precisely which cellular event is associated with PG production. Traditionally, the phagocytic process is considered in three stages: (1) attachment of the particle to the cell membrane; (2) interiorisation (phagocytosis); and (3) fusion of the phagocytic vesicle with intracellular lysosomes (digestion). We show here that PG secretion in response to phagocytic stimuli by macrophages is independent of the first stage, but dependent on engulfment of the particle. Moreover, PG production does not appear to be associated with the process of engulfment *per se*, but with some event following internalisation.

To assess the release of PGs, we prelabelled the culture rabbit alveolar cells (>90% macrophages) with ^{14}C-arachidonic acid (Amersham Searle), as previously described[7]. Analysis of the cell lipids showed that approximately 40–50% of the radioactive arachidonic acid was incorporated into the cell lipids after 1 h of incubation. Most of the radioactivity (68%) resided in phospholipid (41±2% phosphatidyl choline, 17±3% phosphatidyl ethanolamine, and 10±2% phosphatidyl serine and phosphatidyl inositol) ($n = 10$). A small amount (9±2%) was also incorporated into the neutral lipids; only 1–2% of the radioactivity in the cells remained in free fatty acid form. The pattern of incorporation was similar to our previous observation[7].

It has been also shown that concanavalin A (Con A)-treated red blood cells (RBCs) attach to the macrophages, but are not engulfed[9]. We took advantage of this model to study the attachment phase of phagocytosis. As shown in Fig. 1*a*, the Con A-treated RBCs bound to the alveolar macrophages to form rosettes, but none was engulfed at the end of the incubation period (3 h). The PG production of these macrophages was no different from that of the unstimulated group (Fig. 2*a*). However, when anti-rabbit RBC antiserum was added to the culture media together with rabbit RBCs, the latter were rapidly interiorised (Fig. 1*b*). Accordingly, the PG secretion showed a 60% increase compared with the control group (Fig. 2*a*). Neither RBCs nor antiserum alone caused any increase of PG synthesis by macrophages. Thus, the PG release by macrophages during RBC phagocytosis is not dependent on attachment of the RBCs to the macrophage membrane, but depends on the engulfment of the RBCs by these phagocytic cells. This result differs from observations of Goldstein *et al.*[10] in other phagocytic cells such as granulocytes. These cells, once rendered incapable of ingesting particles by treatment with cytochalasin B, generated comparable amounts of thromboxane B_2 to untreated, normal cells.

PG release following RBC phagocytosis by rabbit alveolar macrophages was predominantly PGE_2 (38±4% of total PG released), although PGD_2 (14±2%), $PGF_{2}\alpha$ (25±2%), and

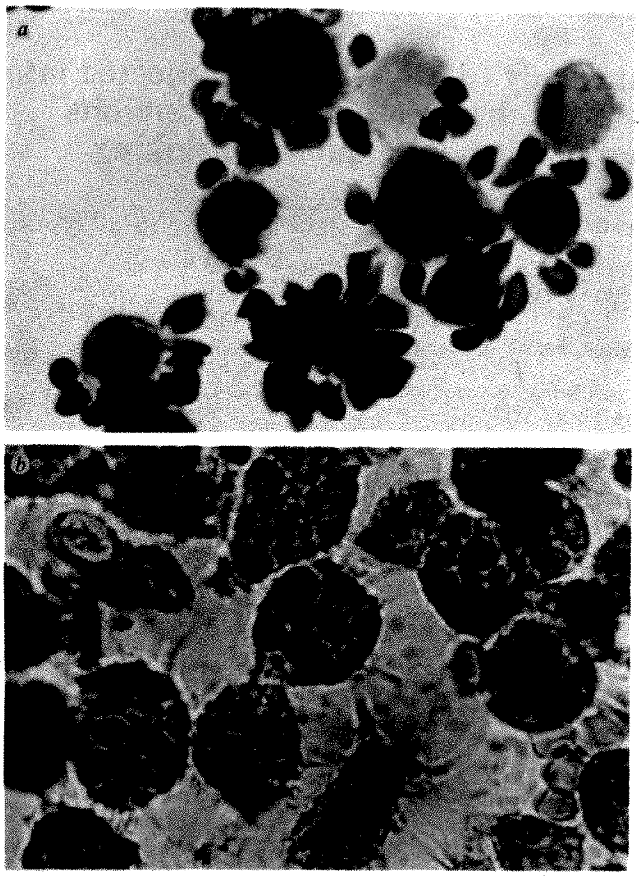

Fig. 1 *a*, Attachment of Con A-treated RBCs to cultured alveolar macrophages. RBCs were collected, washed twice with phosphate-buffered saline (PBS) (*p*H 7.4), resuspended in PBS containing 0.7 μg ml^{-1} Concanavalin A (Sigma), and incubated at 37 °C for 30 min. The RBCs were then washed twice with PBS and 1×10^7 RBCs were added to cultured alveolar macrophages (5×10^6 cells ml^{-1} on each plate). At the end of experimental period, the cells were fixed *in situ* with 10% formalin and stained with haematoxylin and eosin (H and E). *b*, Engulfment of RBCs by cultured alveolar macrophages. The anti-rabbit RBC antiserum was prepared from mice by a modified method of Rabinovitch[11]. A group of 10 mice were injected twice intravenously with 0.2 ml of 10% rabbit RBCs suspended in PBS. The interval between injections was 30 d, and the mouse serum was collected 6 d after the second injection. Fresh rabbit RBCs were collected and washed twice with PBS and 1×10^7 cells were added to alveolar macrophages (5×10^6 cells per plate) in the presence of anti-rabbit RBC antiserum. The cells were fixed *in situ* with 10% formalin at the end of experimental period and stained with H and E stain.

6-keto PGF$_{1}\alpha$ (22±3%) ($n=5$) were also detected in the medium, with a profile very similar to that of zymosan-stimulated alveolar macrophages[7,8].

Our previous work also showed that cytochalasin B, an inhibitor of phagocytosis, when given to zymosan-treated macrophages, increased PG production instead of inhibiting it[7]. In the absence of zymosan, however, cytochalasin B exhibited no effect on PG production[7]. One possible explanation for this paradoxical phenomenon is that PGs are produced during particle–membrane interaction, and that by inhibiting engulfment of the particles, cytochalasin B prolongs the attachment phase, that is, the particle–membrane interaction, thus enhancing PG production. If this hypothesis obtained, we should be able to see a stimulatory effect of cytochalasin B on PG production, when Con A-treated RBCs were used as a stimulus for particle attachment. As shown in Fig. 2*a*, this hypothesis is clearly false. There was no increase of PG secretion by macrophages when cytochalasin B was added to Con A-treated RBCs. However, when RBCs were engulfed in the presence of anti-RBC antiserum, a facilitatory effect of cytochalasin B on PG secretion was clearly demonstrated. This increment was demonstrable at both doses that inhibited phagocytosis (2 μg ml^{-1} and below 0.5 μg ml^{-1}). Therefore, we have shown that: (1) the PG stimulating effect of cytochalasin B was not a result of inhibition of phagocytosis or prolongation of particle–membrane interaction; and (2) the stimulatory effect of cytochalasin B was dependent on the interiorisation of the phagocytosed particles.

Finally, we investigated the relationship between particle engulfment and PG secretion. Zymosan was used instead of

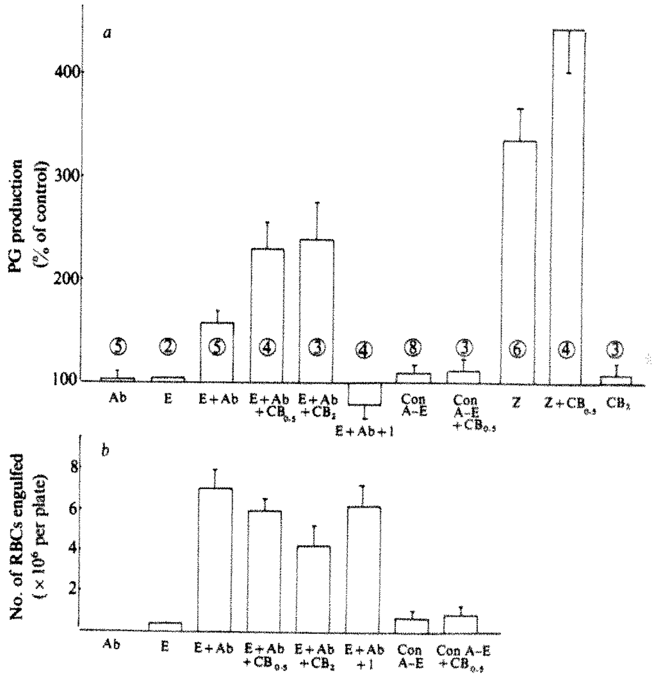

Fig. 2 *a*, ^{14}C-PG production by rabbit alveolar macrophages in response to different stimuli. Rabbit alveolar macrophages were collected as previously described[7] (5×10^6 cells ml^{-1} on each plate). After overnight incubation, the cells were changed to serum-free Dulbecco's modified Eagle's medium (DMEM). ^{14}C-arachidonic acid (specific activity 55 mCi mmol^{-1}, Amersham Searle) was added to the cultures as the Na salt at a concentration of 1×10^6 c.p.m. ml^{-1}. The cultures were incubated for 1 h to label the cell lipids. Cells were washed twice with DMEM, different stimuli were added to the cells and they were incubated for 2.5 h in DMEM containing penicillin (100 μg ml^{-1}), and streptomycin (100 μg ml^{-1}). At the end of the experimental period, the medium was collected, acidified to *p*H 3.5 with formic acid, extracted twice with an equal volume of ethyl acetate, dried and applied to silica gel G plates. The solvent system used for separation of PGs was the organic phase from ethyl acetate : water : iso-octane : acetic acid, 110:100:50:20 (ref. 12). Zones corresponding to various PG standards (provided by Dr John Pike, Upjohn, Kalamazoo) were scraped and counted in a liquid scintillation counter. The total PG release (sum of 6-keto PGF$_{1}\alpha$ + PGF$_{2}\alpha$ + PGE$_2$ + PGD$_2$) was calculated and expressed as the percentage of the control value (unstimulated cells). E, PBS-washed rabbit erythrocytes (1×10^7 cells per plate); Ab, anti-rabbit RBC antiserum (25 μl ml^{-1}); CB$_{0.5}$, cytochalasin B (0.5 μg ml^{-1}); CB$_2$, cytochalasin B (2 μg ml^{-1}); Con A-E, ConA (0.7 μg ml^{-1}) treated RBCs (1×10^7 cells per plate); Z, zymosan (1 mg ml^{-1}); I, indomethacin (2 μg ml^{-1}). The encircled numbers indicate the number of experiments performed (mean±SEM indicated). *b*, Intracellular haemoglobin (Hb) content of macrophages. At the end of the experimental period (2.5 h), the medium was removed and NH$_4$Cl in saline (8.3 mg ml^{-1}) was added to cells to lyse the extracellular RBCs. The medium was again removed, the macrophages were scraped off to a test tube, and Hb content was determined by the cyanmethaemoglobin method[13]. A series of standards of different concentrations of RBCs were used and the Hb content in cells were expressed as number of RBCs in each dish of macrophages (5×10^6 cells ml^{-1} on each plate). (Mean±s.e.m. indicated.)

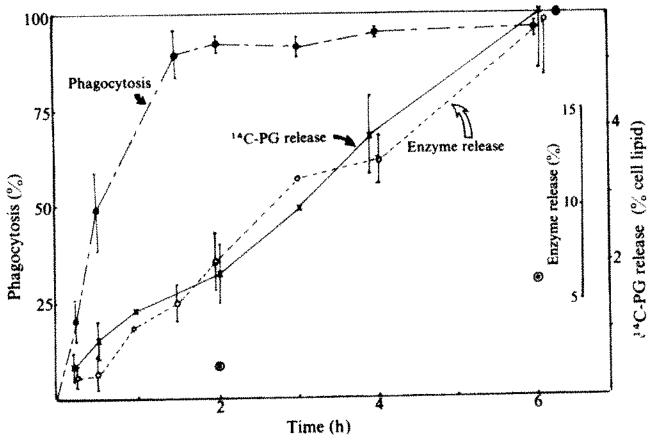

Fig. 3 Time course of phagocytosis of zymosan, lysosomal enzyme release, and PG production by alveolar macrophages. Zymosan was added at time zero, and media were collected at different time intervals for analysis of acid phosphatase activity and PG production. Cells were fixed with 75% alcohol and stained with PAS reaction and the percentage of cells containing two or more zymosan particles (magneta colour by PAS stain) was recorded. The total PG production was determined by radiochemical techniques as described in Fig. 2*a*, and is expressed as percentage of total cell lipid count. The acid phosphatase activity was determined on both medium and cells by a modification of the procedure of Leaback[14]. To determine the intracellular enzyme content, the cells were scraped off the plates into 0.5 ml of 0.1% Triton X-100 in water using a rubber policeman. Aliquots of the cell lysates were diluted 50 times with 0.2 M sodium acetate buffer (*p*H 5). The media were diluted five times with the same buffer. Thirty μl of the diluted medium or cell lysate were added to 70 μl of 4-methylumbelliferyl phosphate (0.05 mg ml^{-1}) in acetate buffer and incubated at 37 °C for 15 min. The enzyme reaction was terminated by adding 1.9 ml of 0.02 M phosphate buffer (*p*H 10.7). The fluorescence was measured in a fluorimeter using a Corning 5860 primary filter and Corning 3387 and 5543 secondary filters. The release of enzyme is expressed as percentage of intracellular acid phosphatase released into the medium (mean + s.e.m., $n = 3$). ×; ^{14}C-PG production; O; percentage of intracellular acid phosphatase released into the medium after zymosan stimulation; ⊚; percentage of intracellular acid phosphatase released into the medium by unstimulated cells; ●, percentage of cells phagocytosing zymosan particles.

RBCs, due to its potent stimulatory effect. As shown in Fig. 3, PG release did not temporally follow the particle engulfment, but exhibited a time lag. Its time course, however, followed closely that of the release of lysosomal enzymes. Furthermore, we have previously shown that cytochalasin B, which increased zymosan-induced release of PGs, also increased that of lysosomal enzymes[7]. Hence, it seems that the release of PGs by alveolar macrophages during phagocytosis is not associated with either the attachment or engulfment phases, but it is concomitant with a much later event, which takes place after the particles have been internalised.

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Antibody to Thy-1 antigen injected into rat hypothalamus selectively inhibits carbamyl choline induced drinking

Curtis A. Williams

Division of Natural Sciences, State University of New York,
College at Purchase, Purchase, New York 10577

James Barna & Nicole Schupf

Department of Psychology, Manhattanville College Purchase,
New York 10577

The Thy-1 differentiation antigen on the cell surfaces of mouse and rat thymocytes, lymphocytes[1–4], epidermis[5], developing skeletal muscle[6], and brain[1,3,4] has not yet been assigned a function. In rat and mouse brain, Thy-1 reaches adult levels at about 3 weeks of age from minimal or variably low levels at birth[3,7–9]. *In vitro* studies of fetal or newborn cerebellar neurons have shown that granule cells and some large multipolar cells become Thy-1 positive during culture[10,11]. All dorsal root ganglion neurones in culture express Thy-1[11,12]. The antigen appears to be enriched in synaptosomal fractions[13,14] but Thy-1 has recently been demonstrated on the surface and dendrites of microdissected neurons of adult rat brain[15]. In the brain Thy-1 has been thought to be a neuronal antigen, but some cerebellar astrocytes become Thy-1 positive after prolonged culture[16]. The transient or developed occurrence of the Thy-1 glycoprotein on selected cell surfaces would suggest that it mediates an event associated either with differentiation or with a differentiated function. The recent report by Dulbecco *et al.* that monoclonal hybridoma antibody against Thy-1.1 (aThy-1.1) blocks a differentiation event in culture of a rat mammary carcinoma cell line[17] is, the first demonstration of a functional link between the molecule and a specific developmental process. We consider brain Thy-1 to be a differentiated adult expression, however, and we report here that injecting antibody to Thy-1 antigen into the rat hypothalamns caused selective inhibition of carbamyl choline induced drinking.

Direct chemical stimulation of the perifornical hypothalamus with the α-adrenergic agonist noradrenaline (NA) elicits food intake in sated rats. Stimulation of the cholinergic system at the same site with the acetylcholine analogue carbamyl choline (CC) elicits a sustained drinking response in water-replete rats[18–21]. If the effect of focal antibody treatment is a result of reaction with antigens characteristic of differentiated neuronal function, then a selective potentiation or inhibition of the eating or drinking response to an exogenous transmitter analogue might be anticipated.

Monospecific antibody against Thy-1 antigens (aThy-1) are produced by hydridising mouse myeloma and immune spleen cells, cloning the hybridoma and culturing as a tumour *in vivo*. Hybridoma aThy-1.1 (T32B11) mouse serum, cytotoxic (complement dependent) for AKR mouse thymocytes at a dilution of 1×10^{-5}, and a monoclonal aThy-1.2 (F7D5), cytotoxic for C57BL mouse thymocytes at a dilution of 1×10^{-6} (ref. 22), were kindly supplied by Dr E. A. Clark. Both antisera were included in the study by Dulbecco *et al.*[17] of the effect on the differentiation of mammary cell cultures. Both were tested in our laboratory by indirect immunofluorescence for specific binding to mouse and rat thymocytes. aThy-1.2 bound to C3H cells but not to AKR or rat cells; aThy-1.1 bound to both AKR and rat cells but not to C3H cells, confirming the designated specificity of these antibody preparations. The antibody is not thought to react with carbohydrate determinants since the brain Thy-1 carbohydrate has a different composition from that of the thymocyte Thy-1[23–25].

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Twenty-four male Charles River CD rats were implanted with chronic unilateral cannulae aimed at the left perifornical hypothalamus. Following surgery and a 2-week recovery period, the animals were divided into two groups to be tested with NA (6 μg) and physiological saline (PS), or with CC (0.8 μg) and PS to determine baseline eating and drinking responses to chemical stimulation. Drug doses were chosen to produce a vigorous behavioural response. Tests were performed at 4-day intervals in a counterbalanced schedule to detect any sequence effects. Half of the animals received the drug (NA or CC) and half received PS on the first day. In the next test the animals were injected with the other substance. All tests were performed at 4 pm, 2 hours before the onset of the dark period of the light–dark cycle. To initiate behavioural arousal and ensure maximal satiation at the start of the test, each rat was given fresh food and water 30 min before the test period. Immediately after this presatiation period, the animals were removed from their home cages, injected with 1 μl of the test solution (NA, CC or PS) and returned to the cage which contained a fresh amount of premeasured food and water. After 1 hour the remaining food was removed from the cage and, with the spillage, weighed to the nearest 0.1 g to determine food intake. Water intake was read to the nearest 1 ml.

The effect of aThy-1.1 on drug-induced behaviours was determined using the same counterbalancing procedure. Tests were performed at weekly intervals. On test days half of the animals in each group received two 1-μl intrahypothalamic injections of aThy-1.1 at 10 and 10:30 am, 6 hours before the chemical stimulation and consumption test period. For injection into the brain site aThy-1.1 serum was diluted fivefold to 14 μg protein per μl since protein concentrations of 25–35 μg μl^{-1} at this site sometimes gave nonspecific effects on consummatory behaviours.

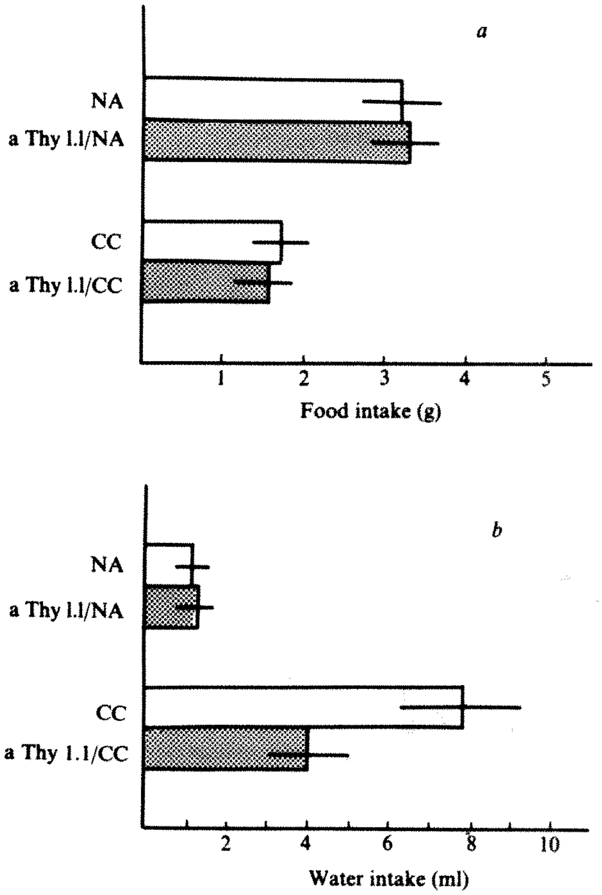

Fig. 1 Drug-induced eating (*a*) and drinking (*b*) modified by intrahypothalamic administration of monoclonal antibody T32B11 to Thy-1.1 antigen (aThy-1.1). Open bars are predetermined drug baseline consumption data. Stippled bars are drug-stimulated consumption data following aThy-1.1 treatment (serum diluted 1/5) administered 6 h before injection of drug into the perifornical hypothalamus. Noradrenaline (NA), 6 μg, and carbamyl choline (CC), 0.8 μg, were administered in 1 μl. ($n = 12$.) Error bars show ±s.e.m.

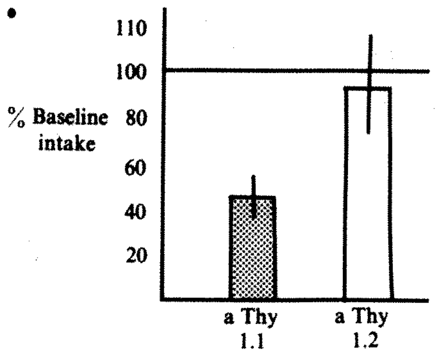

Fig. 2 Comparison of the effects of intrahypothalamic monoclonal antibodies T32B11 (aThy-1.1) and F7D5 (aThy-1.2) on carbamyl choline (CC) stimulation. Data are expressed as percentage of water intake by carbamyl choline (0.8 μg in 1 μl)-stimulated animals following treatment with aThy-1.1 or treatment with aThy-1.2 (serum diluted 1/5) 6 h before injection of drug into the perifornical hypothalamus. ($n = 12$.) Error bars show ±s.e.m.

Figure 1 shows that NA elicited excessive eating while CC elicited excessive drinking, confirming the work of other investigators on the adrenergic and cholinergic regulation of appetitive behaviour at this site[18–21]. Drinking after NA injection and eating after CC injection did not differ significantly from consumption in saline control conditions. Following treatment with aThy-1.1 antibody the drinking response to CC was significantly reduced ($P < 0.02$), whereas neither the drinking nor the eating responses in any other treatment conditions were significantly altered. Repeated baselines with CC indicated complete recovery of the drinking response by 3 days.

These animals were also tested with aThy-1.2, specific for the alloantigenic variant not found in the rat. This was done to control for the possibility that the selective reduction of CC induced drinking was a nonspecific effect, either of some mouse serum component or a hybridoma secretion other than antibody. aThy-1.2 was used at the same protein concentration as aThy-1.1, but only CC-stimulated drinking was tested since that was the only behaviour affected by aThy-1.1. Figure 2 shows the effects of aThy-1.1 and aThy-1.2 on CC-stimulated drinking. Treatment with aThy-1.1 reduced CC-stimulated drinking by $>50\%$, whereas the reduction of mean water intake observed after treatment with aThy-1.2 was not significantly different from the CC baseline. Although aThy-1.1 and aThy-1.2 were allotype-specific, the effect of the high serum concentration of the initial experiment might have been due to some other antibody or substance in the aThy-1.1 serum. The animals were tested, therefore, in groups of eight at aThy-1.1 dilutions of 100, 500, 5,000 and 20,000, alternating with repeat baseline determinations at weekly intervals. Six animals not consistently responding to CC stimulation in three baseline determinations were eliminated from this study. aThy-1.1 diluted 100- and 500-fold significantly reduced the drinking response to CC stimulation ($P < 0.01$). It is probable, however, that dilutions higher than 500 will show a significant effect in larger groups. Because of the activity of aThy-1.1 at 500 dilutions, equivalent to less than 1 pmol of immunoglobulin, we concluded that the effect was Thy-1 specific.

These experiments suggest that the Thy-1 antigen in the rat hypothalamus is distributed in such a way that its reaction with antibody results in a blockade of cholinergic function. The distribution of Thy-1 by structures and brain regions[1,9,15,26] is not inconsistent with a link between Thy-1 and cholinoception. However, the detection of Thy-1 over the entire surface of dorsal root ganglion neurones, which are thought to have only dendritic innervation[11,12], suggests that Thy-1 localisation is not exclusively associated with synapses. Others report that brain Thy-1 is predominantly in the synaptosomal fraction[13,14]. Presynaptic Thy-1 has been demonstrated on aminergic synaptosomes by complement-mediated cytolytic release of transmitters[27], the antigen might still be associated with cholinoceptic modulation of normal release mechanisms.

Our experiments indicate no association of rat brain Thy-1 with α-adrenergic function, at least none which can be blocked by an antibody to Thy-1.1. In an earlier study using the same behavioural test we found complete suppression of NA-induced eating by two different rabbit antisera against a synaptic membrane fraction (aSM). No effect on CC-induced drinking was detectable with aSM. Conversely, a rabbit antiganglioside serum (aGS) greatly reduced the drinking response to CC without affecting NA-induced eating[28]. That study represents an important functional and methodological control even though the target antigen for aSM is not known.

The similar effects of aThy-1.1 and aGS on cholinoceptive function need not imply a common target antigen. Nor is it likely that gangliosides are antigenic determinants of Thy-1 antigens. The aGS we used (preparation 2229 supplied by Dr M. M. Rapport) was raised against whole beef brain gangliosides and it is reported to react with GM_1, G_{D1b} and asialo-G_{M1}, but not with G_{D1a} or G_{T1} gangliosides[29]. It is possible that some antibody in this antiserum cross-reacts with a carbohydrate determinant present on rat brain Thy-1 antigen. To test this possibility, we isolated the IgG fraction of the aGS serum on a staphylococcal protein A-Sepharose 4B column[30]. By radioimmunoassay with ^{125}I-protein A, we find significant binding of aGS2229–IgG to purified rat brain Thy-1 antigen (supplied by Dr A. F. Williams). Consistent with the different carbohydrate composition reported for rat thymocyte Thy-1[23,24], we are unable to demonstrate binding of aGS2229 immunoglobulin to rat thymocytes by indirect immunofluorescence.

These experiments have tentatively assigned a functional correlate to the Thy-1 antigen. Determination of the mechanisms by which aThy-1.1 causes behavioural alterations remain to be investigated. As full responsiveness was recovered in 3 d or less, it is unlikely that the effects are caused by complement-mediated cytotoxic lesions. More likely is the proposal of Barclay *et al.*[23] and Williams *et al.*[24], that the protein mediates a common and special cellular function wherever the Thy-1 antigen is found; the carbohydrate, which may vary from one tissue to another, contributes a specificity to the cell's reactions with other cells and with substances in the extracellular environment. The binding of aThy-1.1 to the protein may affect the function of the molecule by steric hindrance of the carbohydrate specifying function, or by causing a conformational constraint in the protein, or by cross-linking the complex into a nonfunctional array in the cell surface.

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Respiratory oscillations in arterial carbon dioxide tension as a control signal in exercise

D. M. Band

Sherrington School of Physiology, St Thomas's Hospital Medical School, London, UK

C. B. Wolff & Jane Ward

Physiology Department, Guy's Hospital Medical School, London, UK

G. M. Cochrane & J. Prior

Department of Medicine, Guy's Hospital, London, UK

We have monitored oscillations in arterial *p*H (of respiratory frequency) in normal man at rest and during exercise. The *p*H oscillations are known to reflect respiratory oscillations in arterial carbon dioxide tension generated at the lungs. We have found that the *p*H oscillations increase in their upslope and downslope during exercise. This means that oscillations in arterial carbon dioxide tension can be considered as a control signal.

During exercise there is a considerable increase in the ventilation of the lungs which matches the increase in the oxygen requirement of the body and the increase in carbon dioxide production. The ventilatory increase is adequate to keep the levels of oxygen and carbon dioxide reasonably constant in the lungs and also therefore in the arterial blood. How the ventilation is so precisely adjusted to meet the need for oxygen uptake [$\dot{V}O_2$] and carbon dioxide clearance [$\dot{V}CO_2$] has remained obscure in spite of our knowledge of receptors sensitive to altered levels of arterial tensions of both oxygen (the peripheral arterial chemoreceptors) and carbon dioxide (principally the central—medullary—chemoreceptors). Because the arterial levels of oxygen tension [P_aO_2] and carbon dioxide tension [P_aCO_2] do not change much in exercise, Yamamoto suggested that P_aCO_2 oscillations of respiratory frequency might be present and, theoretically at least, would convey information as to $\dot{V}CO_2$ (ref. 1). For P_aCO_2 oscillations to be a reasonable candidate as a control signal in exercise they must get bigger—or at least steeper. The peripheral arterial chemoreceptors are now known to have a high dynamic sensitivity to P_aCO_2 oscillations in the anaesthetised cat[2], although for technical reasons the P_aCO_2 oscillations are measured indirectly, as changes in acidity, with a fast response *p*H electrode. Although direct measurement with a PCO_2 electrode would be preferable none respond sufficiently fast. Even the fast response *p*H electrode

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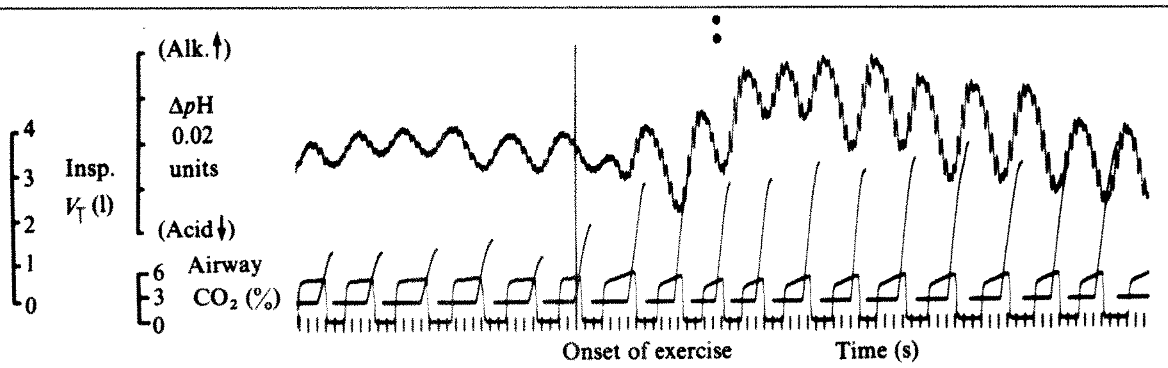

Fig. 1 Photographic record of arterial *p*H oscillations before and after the onset of exercise (40 W) in a healthy subject (C.B.W.). The records below the arterial *p*H record, monitored by means of a left brachial artery cannula, are inspiratory (Insp.) tidal volume (V_T), the airway carbon dioxide concentration, and lines showing the passage of time in seconds. VCO_2 and the average of upslope and downslope of the *p*H oscillations (d*p*H/d*t*) increase 2.7 times with exercise. $\dot{V}CO_2$ increases from 304 to 827 ml min^{-1} BTPS; d*p*H/d*t* from 4.41×10^{-3} to 11.95×10^{-3} *p*H units per second.

systems have so far been highly vulnerable to movement, with excessive electrical noise on any attempt to use them for the indirect measurement of P_aCO_2 oscillations in exercise.

Most of the electrical interference problems have been avoided by linking a fast response *p*H electrode system to a small radio transmitter, while monitoring arterial *p*H from subjects exercising on a bicycle ergometer. This has made possible transmission of the signal to a remote receiver. It has therefore been possible to monitor respiratory oscillations of the arterial *p*H in exercise. We found (Fig. 1) that the *p*H oscillations become steeper during exercise, as postulated if P_aCO_2 oscillations are to be considered as a possible control signal. The change in the form of the oscillation became apparent within 10 s of the onset of exercise.

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Spread of activation and desensitisation in rod outer segments

P. A. McNaughton, K.-W. Yau & T. D. Lamb

Physiological Laboratory, Downing Street, Cambridge, UK

Visual transduction in vertebrate photoreceptors is thought to involve a diffusible internal transmitter which links photon absorption in disk membranes to the conductance decrease in the plasma membrane of the outer segment[1–5]. The desensitisation of the photoresponse which occurs during and after illumination may also involve a diffusible substance, because a photon absorbed in one disk can desensitise the cell's response to a subsequent photon absorbed in a different disk[6]. Although both activation and desensitisation spread longitudinally from the site of photon absorption, neither spreads over the entire length of the outer segment[7,8]. We report here experiments which indicate that, with dim illumination, both effects decline to be half-maximal in less than 8 μm from the site of light absorption.

A single toad rod outer segment was drawn into a closely fitting glass pipette by a recently developed technique[9] and its membrane current was measured. Narrow transverse slits of light could then be positioned as desired along the outer segment, allowing investigation of the spread of activation and desensitisation from the site of light absorption.

In Fig. 1*b* the peak current collected by the pipette, scaled by the flash intensity, is plotted as a function of the displacement of a narrow slit of light from the distal tip, for the cases in which the outer segment was almost completely (●) or only partly (□) sucked into the recording pipette, as indicated schematically in (*a*). In this way curve 2 represents a profile of recorded current as the stimulus was moved off the recording pipette, while curve 1 is a control representing the current collected from the same parts of the outer segment when it was almost completely drawn into the pipette. The shapes of the responses were similar at all stimulus positions except when the slit was some distance off the pipette tip. In this case the intensity had to be substantially increased to give a measurable response, which then exhibited a small (less than 0.5 pA) rapid inward component preceding the normal outward current change. This inward transient might be due to the unstimulated part of the outer segment acting as a current sink. When scaled by flash intensity this negative component was very small on the scale of Fig. 1.

This figure illustrates that activation caused by illumination at one point does not spread over the entire outer segment. For example, stimulation of the point on the outer segment 40 μm from the distal tip gave a measured response which was about 40 times smaller when that part of the cell was just outside the pipette than when it was inside.

The ratio of the two profiles of recorded current, plotted in Fig. 1*c*, gives a measure of the current collected by the pipette as the slit is moved off the tip, corrected for non-uniformities in the sensitivity of the outer segment. If the pipette were a perfect step collector of current (that is if it collected all the current from the region of the cell inside the point of narrowest constriction and none from outside), then the profile in Fig. 1*c* would represent the integral of the membrane current density from the tip of the outer segment to the constriction. Hence the spatial derivative (broken curve) would represent the spread of activation from the narrow line source of illumination; its width at half-height is 15 μm (arrow). This profile is considerably broader than the spread of the stimulating light. Unfortunately we have no direct measure of the collecting efficiency of the pipette, as would be needed to correct the spread function, and we can say only that the curve probably represents an upper limit to the actual spread

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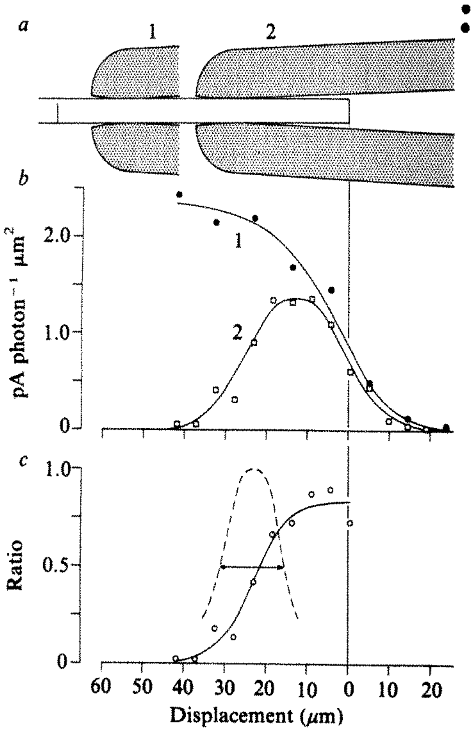

Fig. 1 Spread of activation in a rod outer segment from *Bufo marinus*. *a*, Scale diagram of the outer segment almost completely (1) and partly (2) drawn into pipette. *b*, Recorded current, divided by flash intensity, as a function of displacement of a transverse slit from the tip of the outer segment, for the two positions of the cell. Nominal slit width 6.9 μm. Saturating response of the rod to diffuse light was 18 pA. In this and subsequent figures 20 responses were averaged and flash intensity was adjusted to give approximately 2 pA response; 20-ms flash; 500-nm light plane polarised perpendicular to the long axis of the outer segment; temperature 20°–24 °C. *c*, Ratio of profiles in (*b*); ○ are the ratio of squares (□) to the curve 1 near the filled circles (●); broken curve is the normalised spatial derivative of solid curve. The right hand parts of the curves in (*b*) do not coincide, possibly because of slight movement of the tip of the cell during the experiment. For this reason the ratio has not been plotted beyond 0 μm.

of activation. We believe that this profile has not been substantially narrowed by the presence of current sinks in the unilluminated region because, as discussed above, such sink currents appear to be small. Our upper limit is in broad agreement with a previous estimate of spread in rat rods of 12 μm from the point of absorption[7].

A lower limit can be obtained from the ratio of the amplitude of the single photon event of about 1 pA (ref. 10) to the amplitude of a saturating response of about 30 pA (ref. 9). This indicates that at least 1/30 of the outer segment plasma membrane must be affected by a single isomerisation, so that for an outer segment 60 μm long activation must spread over a longitudinal region of at least 2 μm, corresponding to 1 μm either side of the point of photon absorption.

Figure 2 shows that desensitisation is also localised. Narrow slits were used for both the brief test flash and the steady adapting light, and each was presented near either the base or tip of the outer segment. The heavy traces are averaged responses to the test flashes presented during adapting illumination, while the lighter traces are controls in which the same flashes were presented in darkness. In (*a*) and (*d*) the test and adapting slits were superimposed at the base and tip respectively, while in (*b*) and (*c*) the test and adapting slits were at opposite ends of the outer segment, separated by approximately 30 μm. When they were superimposed the response to the test flash was substantially smaller and faster, but when they were at opposite ends of the outer segment the test response was unaffected by the adapting light. This experiment shows that a light which locally desensitises by three- or fourfold has negligible adapting effect at a distance of 30 μm. On all occasions when substantial desensitisation was observed it was accompanied by accelerated response kinetics, suggesting that the reduction in response amplitude was not due solely to local saturation of light-sensitive channels.

The extent of spread of desensitisation is examined in another cell in Fig. 3. Here desensitisation is expressed as the ratio of sensitivity in darkness to sensitivity in the presence of the steady adapting slit, rather analogous to a plot of threshold elevation in psychophysical experiments. Assuming that the measured profile is symmetrical (broken curve in Fig. 3), it has a width at half-height of 8 μm (arrow). The desensitisation has been plotted in this way in an attempt to obtain a linearised measure of the effect of adapting light. With diffuse illumination, toad rod sensitivity shows Weber–Fechner behaviour[6,11,12], defined by

$$\frac{S_F^D}{S_F} = 1 + \frac{I}{I_0} \qquad . \qquad (1)$$

where S_F is the sensitivity in the presence of background intensity I, S_F^D is the sensitivity in darkness and I_0 is the intensity that halves sensitivity. Note that the term $(S_F^D/S_F - 1)$ is directly proportional to adapting intensity. If we assume that light generates a diffusible substance whose concentration rises linearly with intensity, and that desensitisation at any point on the outer segment depends on the local concentration of this substance, then we can interpret the profile above the line $S_F^D/S_F = 1$ in Fig. 3 to represent the spread of desensitising substance. For comparison the measured light spread is indicated by the narrower curve in the figure; it has a width at half-height of only 2.6 μm.

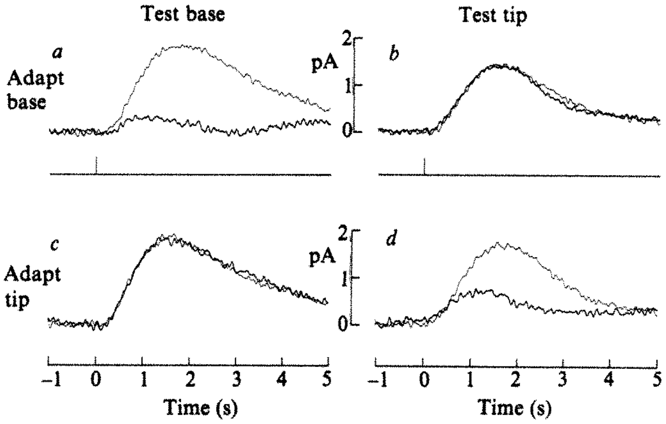

Fig. 2 Localisation of desensitisation. The test and adapting stimuli were narrow slits which could be independently positioned near either the tip or the base (as indicated) of an outer segment drawn fully into the pipette; the two slit positions were separated by about 30 μm. In each part of the figure the heavier trace is the averaged response to the test flash in the presence of the steady adapting slit, and the lighter trace is the control in darkness. Identical stimuli were used throughout; adapting slit gave steady response of about 3 pA at both positions; nominal slit widths 6.9 μm. Saturating response to diffuse light was 20 pA. Responses low-pass filtered at 10 Hz.

Some justification for the linearising procedure adopted in the previous paragraph comes from a comparison between the effects of local and uniform illumination. In Fig. 3 the adapting slit delivered about 7 photoisomerisations per s and the area under the profile of $(S_F^D/S_F - 1)$ was 54 μm. If the same number of photoisomerisations were spread uniformly over the whole outer segment length of 60 μm then the average value of $(S_F^D/S_F - 1)$ would be 54 μm/60 μm = 0.9. Thus the intensity

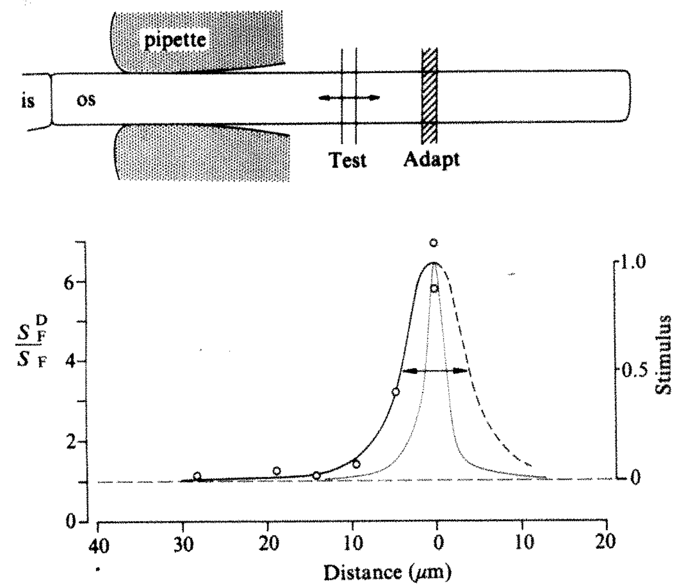

Fig. 3 Spread of desensitisation. Top: diagram of outer segment and light slits on the same scale as the lower abscissa. Bottom: desensitisation, expressed as dark sensitivity (S_F^D) divided by sensitivity (S_F) in the presence of the adapting light (left-hand ordinate, open circles; heavy curve fitted to the points by eye, and assumed to be symmetrical to obtain the broken extension), as a function of distance between the steady adapting slit and the test flash slit (abscissa). The position of the steady adapting light was fixed (0 μm on abscissa) and the test flash was moved to different positions on the outer segment. The thin line shows the normalised intensity (right-hand ordinate) of the adapting slit, determined in another experiment by moving the image of the slit across a small aperture placed in the microscope eyepiece. Both slits had a nominal width of 1.7 μm. The steady light had a nominal intensity of 4.6 photons $\mu m^{-2}\,s^{-1}$, and produced a mean response of 2.2 pA. Saturating response to diffuse light was 16.4 pA.

I_0 of diffuse light required to halve the sensitivity (that is, $S_F^D/S_F - 1 = 1$) would be $7/0.9 = 7.8$ photoisomerisations per s. This is in reasonable agreement with measured values of about 4 photoisomerisations per s[6,11,12].

Preliminary experiments indicate that the integration time[13] of desensitisation resulting from flash illumination is about 3.5 s. Applying this value and equation (1) to the cell in Fig. 3 we estimate that, during this integration time, a single photoisomerisation should on average reduce sensitivity by about 18% (that is $S_F/S_F^D \simeq 0.82$) at the site of light absorption.

We conclude that the spread of activation and desensitisation is restricted mainly to a distance of 5–10 μm along the outer segment from the site of photon absorption. The extent of spread of desensitisation lies between our upper and lower estimates for spread of activation, and it is possible that both effects spread to the same extent, as would be the case if the same diffusible substance were ultimately responsible for both processes.

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Localisation of substance P immunoreactivity in amacrine cells of the retina

H. J. Karten & N. Brecha*

Department of Psychiatry and Behavioral Science,
School of Medicine—Health Sciences Center,
State University of New York at Stony Brook, New York 11794

Since its actions on smooth muscle were first described by von Euler and Gaddum[1], substance P has been isolated and characterised as an undecapeptide[2,3]. The distribution of substance P-like immunoreactivity in both the central and peripheral nervous systems[4,5] and its reported excitatory action on neurones implicate substance P as a possible neurotransmitter and/or neuromodulator[6,7]. Previous studies using bioassay[8,9] and radioimmunoassay[10,11] techniques have reported substance P in retinal extracts. Here we describe the specific immunohistochemical localisation of substance P-like immunoreactivity in a population of morphologically distinct amacrine cells of the pigeon retina. The localisation of substance P-like immunoreactivity and the recent localisation of enkephalin-like[12,13] and somatostatin-like immunoreactivity[14] in other types of amacrine cells suggest that the neuropeptides have a specific role in retinal function.

Details of the methods used for localisation are described in the legend to Fig. 1. Substance P-like immunoreactivity was observed only in the inner nuclear and inner plexiform layers of the retina with both the indirect immunofluorescence and immuno-peroxidase bridge procedures. Specific substance P-like immunoreactivity was observed in the somata of amacrine cells usually located at the border of the inner nuclear and inner plexiform layers (Figs 1*a*, 2). No immunoreactive somata were observed in the outer nuclear layer, the distal inner nuclear layer or the ganglion cell layer. Muller cells were not immunoreactive. Immunoreactive cell processes were observed only in the inner plexiform layer.

The immunoreactive amacrine cells have a single process, which descends directly into the inner plexiform layer and ramifies in a narrow band of the inner plexiform layer corresponding to lamina 3 of Cajal[15]. Substance P-like immunoreactivity was observed in both central and peripheral retinal regions. The immunoreactive amacrine cells in the tangential sections were about 7 μm in diameter and were distributed throughout the retina. Relative to other retinal regions, a greater number of immunoreactive somata were located in the inferior retina and a smaller number of immunoreactive somata were located within the red field of the superior retina.

The staining for substance P seems to be specific, as it was eliminated by incubation of the retinal sections in substance P monoclonal antiserum preabsorbed with 10 μm synthetic substance P (Fig. 1*b*). Moreover, retinal sections incubated in substance P antiserum preabsorbed with 10 μM synthetic neurotensin, Leu^5-enkephalin, Met^5-enkephalin, somatostatin or thyrotropin-releasing factor demonstrated positive immunoreactivity in the same type of amacrine cell, indicating that the substance P antiserum did not react with these other peptides in our incubation conditions. Incubation of retinal sections in either normal or preabsorbed antisera demonstrated nonspecific immunoreactivity in a few ganglion cells located in the peripheral retina.

The present study demonstrates a specific population of substance P-like immunoreactive amacrine cells and their processes in the avian retina. The consistent size of their somata,

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* To whom correspondence should be addressed.

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their location in the proximal inner nuclear layer and arborisation pattern in lamina 3 of the inner plexiform layer suggest that neither bipolar cells nor displaced ganglion cells contain substance P-like immunoreactivity[15,16]. The profile of the substance P-like immunoreactive cell is similar to amacrine cell type 'J' described by Cajal[15] from Golgi material. On the basis of somatic location and arborisation patterns, the substance P-like immunoreactive cells do not seem to overlap with the indoleamine[17] or dopamine[18] containing amacrine cells.

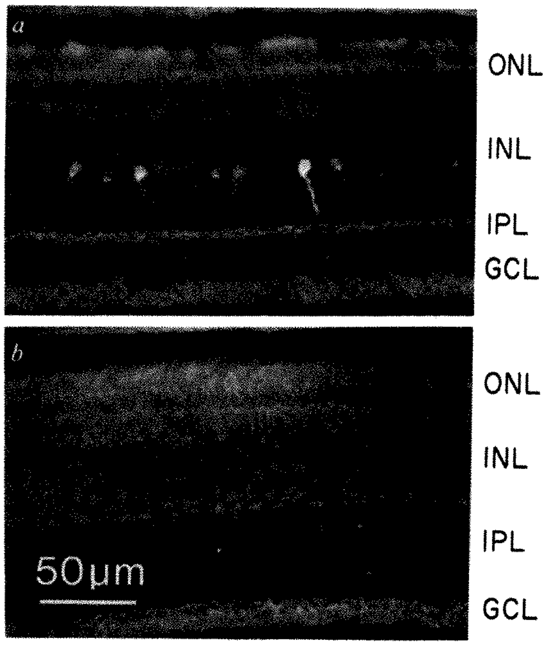

Fig. 1 *a*, Low power photomicrograph of a retinal section incubated in substance P antiserum demonstrating substance P-like immunoreactivity in an amacrine cell and in processes located in the inner plexiform layer. Several other substance P-like immunoreactive somata occur in a different plane of focus. *b*, Low power photomicrograph of a retinal section incubated in substance P antiserum which had been preabsorbed with 10 μM synthetic substance P, demonstrating lack of substance P-like immunoreactivity. ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. Retinal sections for Figs 1 and 2 were prepared as follows. Adult white Carneaux pigeons were deeply anaesthetised with intramuscular ketamine and perfused through the left ventricle with 6% dextran in 0.1 M phosphate buffer (*p*H 7.2) followed by 4% paraformaldehyde in 0.1 M phosphate buffer (*p*H 7.2). During the perfusion the eyes were injected with the fixative. They were postfixed for 4–6 h at 4 °C and stored overnight in 30% sucrose phosphate buffer at 4 °C. Sections were cut perpendicular to the vertical axis of the eye (horizontal plane) at 12 μm with a cryostat or tangential to the retinal surface at 30 μm with a freezing microtome. They were processed for immunohistochemical staining using either the indirect immunofluorescence or the immunoperoxidase bridge techniques previously described[13]. Sections were incubated in substance P antiserum or substance P antiserum which had previously been absorbed with either 10 μM synthetic substance P, neurotensin, Leu^5-enkephalin, Met^5-enkephalin, somatostatin or thyrotropin-releasing factor at a dilution of 1:1,000 for 12–48 h at 4 °C. These experiments were replicated with at least 12 retinae except for the preabsorption series of neurotensin, Leu^5-enkephalin, Met^5-enkephalin, somatostatin and thyrotropin-releasing factor, which were replicated with two pigeon retinae.

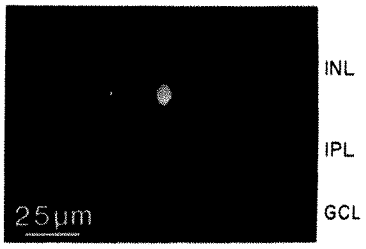

Fig. 2 High power photomicrograph demonstrating a substance P-like immunoreactive amacrine cell.

The localisation and distribution of substance P-like immunoreactive amacrine cells differ from those of enkephalin-like immunoreactive amacrine cells in the avian retina[13]. Enkephalin-like immunoreactivity has been observed in many small amacrine cells, giving rise to a diffuse plexus of processes which ramify in laminae 1 and 3–5 of the inner plexiform layer. In contrast, the substance P-like immunoreactive amacrine cells, although also small, ramify in lamina 3 of the inner plexiform layer. The enkephalin-like immunoreactive amacrine cells are distributed throughout the entire retina, are most numerous in the red field of the superior retina and may be related to the total number of cells in the inner nuclear layer[19]. The distribution of substance P-like immunoreactive amacrine cells, however, does not seem simply to be related to the total number of cells in the inner nuclear layer in as much as the greatest numbers of substance P-like immunoreactive cells are in inferior retinal regions and not the red field. The differential distribution of substance P-like immunoreactivity suggests a histochemical as well as a morphological basis for classification of retinal amacrine cells. Amacrine cells have been classified on the basis of their histofluorescent characteristics and their differential uptake systems for γ-aminoglutamic acid, glycine, choline or dopamine[20–23]. However, the total number of amacrine cells and the distribution of subclasses of amacrine cells in the retina are unknown. Therefore, it is not yet possible to compare the number of substance P-like and enkephalin-like immunoreactive amacrine cells with other histochemically distinct subclasses of amacrine cells.

Recently, several neuropeptides have been found in the retina of different vertebrate species using radioimmunoassay techniques[24–28]. Substance P-like, enkephalin-like, neurotensin-like and somatostatin-like immunoreactivity have been localised in distinct classes of amacrine cells of different vertebrate retinas using the immunohistochemical technique (refs 12–14 and unpublished observations).

In conclusion, specific substance P-like immunoreactivity occurs in a distinct class of retinal amacrine cells. The specific functional role of substance P or any other neuropeptide in the retina is unknown although their localisation in selected populations of retinal cell types suggest they have unique roles in retinal processing.

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Removal of the synaptic target permits terminal sprouting of a mature intact axon

Kenneth J. Muller & Sheryl A. Scott*

Department of Embryology, Carnegie Institution of Washington,
115 West University Parkway, Baltimore, Maryland 21210

When the central nervous system (CNS) develops, neurones send out axons to make contact with appropriate synaptic target cells and then stop growing. If its usual target is missing, an axon may continue to grow until it synapses with a suitable but inappropriate target[1]. This suggests that contact with a synaptic target is important in stopping axonal growth during development. Many classes of neurone in the adult CNS retain a capacity to grow towards denervated targets[2-5], but it is not known whether the synaptic contacts established during development continue to regulate the growth of individual mature, intact axons. This has been a difficult problem to investigate; in vertebrates most studies necessarily involve large populations of neurones, and the most direct approach, removal of a synaptic target, usually damages many neurones, including the axons that are to be studied. We report here a demonstration of target cell influences on the growth of a single mature, intact axon in the CNS of the leech by selectively destroying the axon's synaptic target without injuring the axon itself. Target removal, which itself does not trigger sprouting of intact axons, permits the intact axon to grow at its tip in response to injury of other axonal branches of the same cell.

The system of S-interneurones in the leech has been particularly useful for studies of axonal growth and synapse regeneration[6-8]. In each of the 21 segmental ganglia there is one S-cell; it extends an axon both anteriorly and posteriorly about halfway along the connectives (the axon bundles that link adjacent ganglia) to make an electrical synapse exclusively with the tip of the next S-cell axon[8]. For brevity, we shall use the 'target' of the S-cell axon to denote the neurone with which that axon is synaptically linked. In this sense, each of the two synaptically coupled S-cell axons is the target of the other.

To test whether contact with a synaptic target has any influence on mature intact axons, we selectively killed single S-cells in the nervous system of an otherwise intact leech by injecting the soma of the target cell with a protease (Type VIII, Sigma)[9,10]. This approach was taken because it is not possible to eliminate the target by removing its soma, as the S-cell axon can survive for weeks severed from its cell body. Protease-injected S-cells were rapidly and completely destroyed, with no direct electrophysiologically or morphologically measurable effects on other cells[9,10]. The long-term effects on the uninjured S-cells adjacent to the killed cell were followed by dissecting chains of ganglia from 4 days to 6 months after the operation, impaling the S-cells with microelectrodes, and injecting them with horseradish peroxidase (HRP), a marker that does not cross the S-cell synapse but fills the injected cell[8], or with Lucifer Yellow, a fluorescent dye that does cross between electrically coupled cells[10,11], or with both. We studied 15 neurones in 14 preparations using electrophysiological methods, and stained and examined them with light and electron microscopy.

The S-cell axons that had been coupled to the killed cells were apparently unaffected by the loss of this synaptic contact. The neurones maintained full-calibre axons that extended to the original site of synapse with the killed cell, about halfway along the connectives, and they showed no signs of growth or retraction. The intact axons did not develop recognisable growth cones filled with characteristic smooth membranous reticulum[8,12]. Furthermore, the free axons apparently did not form synapses with other axons in the connectives. Lucifer Yellow dye provides one test for electrical synapses that resemble those made between normal S-cells. When injected into either a normal or a regenerated S-cell, the dye readily diffuses down the axons and selectively fills the adjacent S-cells, crossing into them at the electrical synapse (S.A.S. and K.J.M., in preparation). In contrast, Lucifer Yellow injected into S-cells that were adjacent to a killed cell stopped abruptly at the tip of the free, intact axon midway along the connectives, and filled no other axons (S.A.S. and K.J.M., in preparation). Moreover, no structures resembling electrical[8] or chemical[13] synapses were seen near the tips of such intact axons when viewed in the electron microscope. Thus, an S-cell axon does not seem to be affected when its unique synaptic target is selectively destroyed.

One obvious difference between neurones in developing nervous systems and those in adults that could account for the failure of the adult neurone to respond to the loss of a target is that in the adult the neurone is no longer growing when its target is destroyed. To prompt the adult S-neurone to mobilise its cellular machinery for growth, we injured the cell by severing one of its two principal axons. The target of the neurone's other, intact axon was then removed by injecting that target cell with protease (Fig. 1*a*). A total of 22 intact axons of injured neurones in 12 preparations were examined from 9 days to 6 months after surgery. Identical results were obtained whether a cell's anterior target or its posterior target S-cell was killed.

The severed axon of these cells regenerated normally[8] to form an electrical synapse selectively with the tip of the adjacent S-cell axon in about 1 month, but the response of the intact axon was surprising. Ordinarily, when one S-cell axon is severed, the cell's other, intact axon is not measurably affected by the lesion;

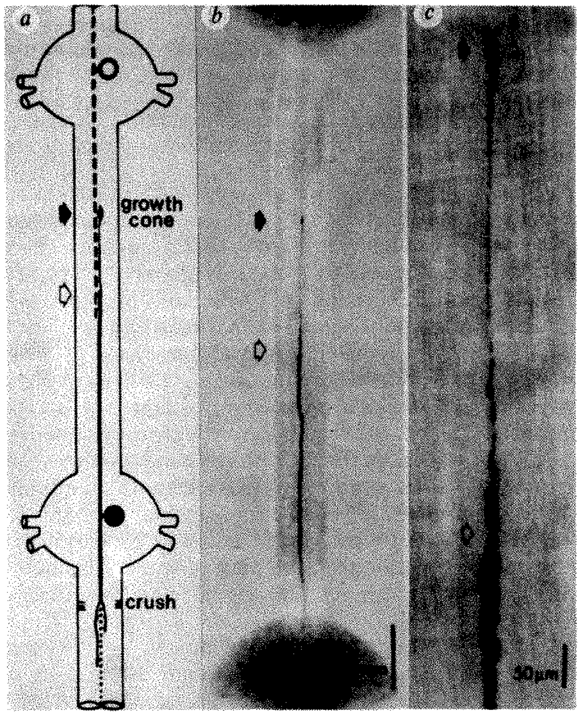

Fig. 1 An intact axon can be made to sprout when its synaptic target is selectively removed. The neurone in the ganglion at top (anterior) was injected with protease, which destroyed the entire cell (dashed line and open circle in *a*). A distant axon of the adjacent S-cell was then severed by crushing the connective (crush). The injured axon regenerated in a normal fashion across the crush and along its severed distal stump (dotted line, *a*); the intact axon of that cell, which had been in synaptic contact with the killed cell near the connective midpoint (open arrow), sprouted a fine process tipped with a growth cone (solid arrow). In *b* the sprouting neurone was marked by intracellular injection of horseradish peroxidase 25 days after its posterior axon (not visible) had been severed. *c*, The sprouted axon is seen at higher magnification, showing the growth cone (solid arrow) extending from the original axon tip (open arrow). This tissue was not cleared so that it could be subsequently examined in the electron microscope.

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*Present address: Department of Neurobiology and Behavior, State University of New York at Stony Brook, Stony Brook, New York 11794.

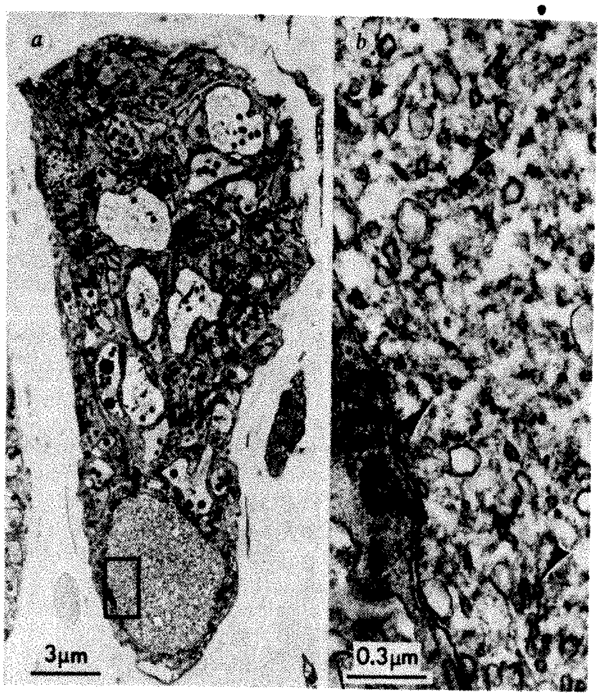

Fig. 2 The axon that sprouts in response to the loss of its target grows along the path of the destroyed cell. The sprouted neurone was marked with intracellular injection of horseradish peroxidase 38 days after the adjacent S-cell had been killed. The electron micrograph in *a* shows a cross-section of the connective cut through the growth cone of the sprouted axon, which is located at the position in the middle connective (Faivre's nerve) that is normally occupied by the killed cell. The growth cone contains much smooth membranous reticulum (arrowheads in *b*, an enlargement of boxed region of *a*) characteristic of the tips of growng axons.

it remains in synaptic contact with its target and does not grow[8]. In striking contrast, the intact axon of the injured neurone sprouted at its tip when its target was destroyed with protease. Most intact axons of injured S-cells eventually grew about half the distance from the original axon tip, which was clearly recognisable (Fig. 1), to the next ganglion; one axon grew as far as the adjacent ganglion. Terminal sprouting was most obvious in cells examined 3–6 weeks after surgery. In these cells a single fine process, enlarged at the tip to a growth cone, extended from the end of the intact axon (Fig. 1). Electron microscopy showed that the growth cones were filled with a smooth membranous reticulum and that they followed a route indistinguishable from that formerly occupied by the killed S-cell (Fig. 2). Although this route might simply have been the path of least resistance, the possibility remains that it was actively selected, as is evidently done by regenerating axons[8].

The sprouted axons stopped growing during the second month after surgery, apparently without establishing any synaptic contacts. Electron micrographs of the tips of the sprouted axons showed that the growth cone together with its content of smooth membranous reticulum disappeared, and that no structures resembling chemical or electrical synapses had formed. Furthermore, Lucifer Yellow injected into sprouted S-cells filled the intact axon to its tip without passing into other axons; this paralleled HRP labelling of the same cells (S.A.S. and K.J.M., in preparation). Under the present circumstances, the sprouted S-cells may have failed to establish synapses in the connectives simply because unoccupied targets were not available. Had the connective itself been injured, the sprouted axons may then have formed synapses with alternative targets[5,14].

In most preparations examined more than 3 months after surgery, the tip of the S-cell axon was not as fine and long as in preparations examined at earlier times, suggesting that sprouts may begin to retract if they fail to find a suitable target. However, we have seen no evidence of a complete retraction of the sprouts; even at the longest times it was clear that a sprout extended from the original tip of the axon, which remained distinct.

Thus, removal of the synaptic target of a mature, intact axon in the CNS of the leech permits the axon to sprout if the neurone itself has been triggered to grow by direct injury to another of its branches. Neither simple elimination of the synaptic target nor injury to another axon of the cell is sufficient to induce sprouting, although if new targets could be made available the neurone might sprout[1,3]. We do not know what aspect of cellular injury prompts terminal sprouting of an intact, denervated axon, nor do we know the signal that stops axonal growth, as the axon does not form synapses. It might be that growth stops when the cell reaches some maximal arborisation, or that all growth ceases soon after the severed axon stops regenerating. The findings reported here suggest the possibility that axonal injury triggers general growth processes in the damaged neurone, like those in developing neurones, expressed only by axonal branches that have lost contact with their targets. Although it is not known how the target exerts its effects on the axon, whether by impulse activity across the synapse, transfer of molecules between the coupled cells, direct physical contact, or some other means, it is clear that in the adult nervous system synaptic contacts can inhibit the growth of intact axons.

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Pirenzepine distinguishes between different subclasses of muscarinic receptors

R. Hammer*, C. P. Berrie, N. J. M. Birdsall, A. S. V. Burgen & E. C. Hulme

Division of Molecular Pharmacology, National Institute for Medical Research, Mill Hill, London NW7, UK

Some antagonists exhibit tissue selectivity in their pharmacological antagonism of muscarinic responses[1]. However, the affinity constants for equilibrium binding of classical antagonists to muscarinic receptors in subcellular preparations have shown only small variations in different peripheral tissues and regions of the brain[2–6]. The binding curves do not deviate significantly from the simple Langmuir isotherm, indicating apparent homogeneity of the receptor population in any given region [5–8]. In contrast, heterogeneity has been detected by agonist binding studies[8,9] but this may arise from different environmental or coupling restraints on the agonist-induced conformational change[10,11] and cannot be taken as evidence for different receptor subtypes. We report here binding studies using a new anti-muscarinic drug, pirenzepine, in which we found heterogeneity of binding that correlates well with the pharmacological activity.

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* Permanent address: Department of Biochemistry, Dr Karl Thomae GmbH, Biberach an der Riss, FRG

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Pirenzepine (LS 519 Cl2), (Fig. 1) inhibits gastric secretion in animals[12] and man[13–15], and there is strong evidence that this effect is governed by an antimuscarinic mechanism[16,17]. It also antagonises the cholinergically stimulated contraction of the smooth muscle of the ileum[18] and urinary bladder[19], blocks salivation, and causes tachycardia[19]. However, in contrast to the action of classical antimuscarinic drugs, there is a wide disparity between the doses of pirenzepine needed to evoke these different effects; whilst effects on gastric secretion are seen at very low doses, higher doses are needed to inhibit salivation, and only with very high doses are effects seen on the smooth muscle of the stomach wall and on the heart[19,20]. Central effects are not significant since the drug is hydrophilic[21] and therefore does not cross the blood barrier to any appreciable extent[22]. In the present study the binding of pirenzepine to muscarinic receptors in homogenates of several peripheral tissues and in different regions of the rat brain has been compared to that of a classical muscarinic antagonist, *N*-methylscopolamine (NMS).

The binding of NMS was determined directly using the radiolabelled ligand ^{3}H-NMS (refs 7, 9) and representative binding curves in the sublingual gland and atria are shown in Fig. 2. There is only a small difference in the two estimates of the ^{3}H-NMS dissociation constant in the two tissues and in all the areas examined the ^{3}H-NMS binding curves are adequately described by a simple Langmuir isotherm having Hill coefficients (H) close to, or not significantly different from 1.0 (Table 1).

Table 1 Pirenzepine and NMS binding in peripheral organs and in brain

Region	Pirenzepine $IC_{50\,cor}$(M)	H	NMS K_d(M)	H
Brain				
Hippocampus	3.4×10^{-8}	0.80*	2.7×10^{-10}	1.05
Cerebral cortex	5.1×10^{-8}	0.73*	3.6×10^{-10}	1.00
Medulla-pons	4.1×10^{-7}	0.97	4.5×10^{-10}	1.04
Glands				
Sublingual	1.1×10^{-7}	0.72*	3.0×10^{-10}	0.91
Submandibular	1.3×10^{-7}	0.78*	2.5×10^{-10}	1.01
Lacrimal	2.0×10^{-7}	0.97	3.2×10^{-10}	1.04
Parotid	2.9×10^{-7}	0.97	2.6×10^{-10}	0.90
Heart and smooth muscle				
Atria	8.3×10^{-7}	0.96	5.9×10^{-10}	0.96
Ileum wall	7.9×10^{-7}	0.96	6.7×10^{-10}	0.96
Stomach wall	1.1×10^{-6}	0.98	5.3×10^{-10}	1.08

All manipulations and assays were performed in 100 mM NaCl, 10 mM $MgCl_2$ 20 mM HEPES (*p*H 7.5). The salivary glands, lacrimal gland and atria were homogenised initially in buffer (Sorvall Omnimix, setting 4, 60 s, 0 °C). The ileum and stomach wall were dissected free of mucosa and homogenised (Ultra-Turrax, setting 6, 2 × 30 s, 0 °C). All initial homogenates of peripheral organs were further homogenised (Potter–Elvehjem, 20 strokes, 520 r.p.m., 0 °C) and filtered through cheesecloth. It was found that the presence of the proteolytic inhibitor, phenylmethylsulphonylfluoride (1 mM) had little effect on the receptor binding properties. The brain regions were only subjected to the Potter homogenisation. For the binding assays the homogenates in appropriate dilutions (~1 mg protein ml^{-1}) were preincubated for 10 min at 30 °C before addition to microcentrifuge tubes containing labelled ligands with or without unlabelled ligands. Incubation was carried out for 20 min (at which time, binding was at equilibrium) at 30 °C and was terminated by centrifugation as previously described[7]. Assays were carried out in quadruplicate and the non-specific binding defined as the radioactivity bound or entrapped in the pellet when the incubation medium contained 10^{-6} M 3-quinuclidinyl-benzilate. Binding curves for NMS were measured directly with the radio-labelled ligand. Binding curves for pirenzepine were derived indirectly from competition studies against 3×10^{-10} M ^{3}H-NMS (peripheral organs), or 10^{-9} M ^{3}H-propylbenzilylcholine (brain regions) as described previously[7]. K_d for NMS were estimated from ^{3}H-NMS saturation curves according to a one-site model using non-linear least squares regression analysis[7]. The IC_{50} values for pirenzepine binding were corrected for the radioligand occupancy shift according to $IC_{50\,cor} = IC_{50}/(1+[C]/K_d)$, where K_d and [C] represent the dissociation constant and the concentration of the radioligand respectively. H values were calculated by linear regression analysis and analysed for statistically significant deviation from unity. The data are, in general, the mean of at least two independent measurements which differed in IC_{50} value by less than 20%.

* Significantly different from 1 ($P < 0.05$).

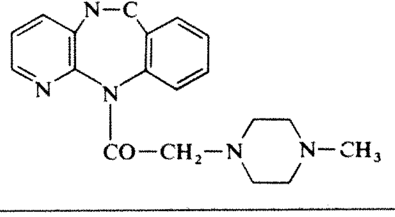
The binding of pirenzepine was determined by the inhibition of receptor-specific binding of ^{3}H-propylbenzilylcholine or ^{3}H-NMS (refs 7, 9). Occupancy concentration curves for pirenzepine binding in preparations from the cerebral cortex, sublingual gland, parotid gland and atria are shown in Fig. 2. Experimental details are given in the legend to Table 1. It is clear that there are large differences in the concentrations of pirenzepine giving 50% occupancy of the receptors in the various regions. The pirenzepine binding curves in the parotid and atria have H-values close to 1.0, whereas the binding curves for pirenzepine in the cortex and sublingual gland deviate significantly from unity (Fig. 2, Table 1) suggesting the presence of sites of more than one affinity[8,9]. Nonlinear least-squares regression analysis shows that the presence of two populations of sites having dissociation constants (K_d) of 2.5×10^{-8} M and $0.7–1.0\times10^{-6}$ M are needed to adequately account for these flat curves. Preliminary direct binding experiments also show that low concentrations of ^{3}H-pirenzepine bind specifically to a high affinity population of sites in the cortex, with K_d of 2.5×10^{-8} M, thereby confirming the indirect measurements (R.H. *et al.*, unpublished results). The pharmacological specificity and the binding properties of the high affinity ^{3}H-pirenzepine sites suggest that they are a sub-class of those labelled by ^{3}H-NMS.

Table 1 summarises a series of pirenzepine binding measurements. The lowest IC_{50} values for pirenzepine were found in the hippocampus and cerebral cortex; both areas exhibited heterogeneous binding curves. Intermediate values were found in the medulla-pons, the salivary glands and the lacrimal gland. The parotid and lacrimal glands appear to contain relatively uniform populations of pirenzepine sites, with K_d of $2–3\times10^{-7}$ M, whilst the sublingual and submandibular glands gave H values significantly less than 1.0, and appear to contain a small proportion of high affinity sites. Heart and smooth muscle were found to contain a homogeneous, low affinity population of pirenzepine binding sites ($K_d \approx 1\times10^{-6}$ M). All the pirenzepine binding data are compatible with the presence of three sites with $K_d \sim 2\times10^{-8}$ M, 2×10^{-7} M and 1×10^{-6} M respectively; an overall variation of 50-fold. In contrast, the affinity for ^{3}H-NMS varied by less than a factor of 3 and further confirms the lack of selectivity for binding of classical antagonists to muscarinic receptors from different tissues; again the lowest values were found in heart and smooth muscle with somewhat higher values in the glands and brain, perhaps demonstrating some analogy with the distribution of pirenzepine affinity constants.

The *in vitro* binding profile of pirenzepine in the peripheral organs strikingly parallels the selectivity of the pharmacological profile of the drug, both in animal tests[19,20] and in clinical trials[14,15,23–25]; even very high doses do not cause tachycardia and only occasionally are mild and transient 'dry mouth' effects reported. We have not yet been able to determine the pirenzepine binding properties of the muscarinic receptors in the parietal cells of the gastric mucosa because of proteolysis and the very low concentration of binding sites.

Whilst muscarinic agonists also exhibit heterogeneity of binding, it does not appear that pirenzepine has agonist or partial agonist activity in pharmacological studies, and in addition, in the brain, the affinity profile of pirenzepine is actually opposite to that of the agonists, which show predominantly high

Fig. 1 Formula of pirenzepine.



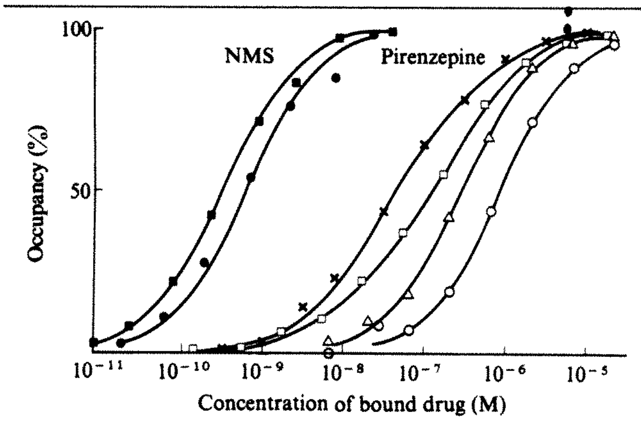


Fig. 2 Occupancy concentration curves for the binding to muscarinic receptors in different tissues of NMS (■ sublingual gland, ● atria) and pirenzepine (× cerebral cortex, □ sublingual gland, △ parotid gland, ○ atria) at 30°C. Experimental details as in the legend to Table 1.

affinity binding in the medulla-pons, and low affinity binding in the hippocampus[5]. Moreover, preliminary experiments show that the binding of pirenzepine is not affected by guanosine triphosphate in tissues, such as heart, where muscarinic agonist binding is modulated by guanine nucleotides[26]. Such behaviour is characteristic of an antagonist.

Previous evidence has favoured the hypothesis that the receptor subtypes detected by agonists do not differ in their intrinsic structure but rather in the molecules associated with them; this could equally be the explanation for the subtypes detected by pirenzepine, but we cannot yet exclude the possibility that pirenzepine senses small intrinsic differences in receptor structure.

It is evident that pirenzepine is able to discriminate differences in the subclasses of muscarinic binding sites which are not detected by classical antagonists and which make its selective antimuscarinic action possible. While the pattern of selectivity exhibited by pirenzepine favours blockade of the gastric secretogogue action of muscarinic agonists as compared with the effects on the heart, it is possible that drugs will be found with the converse selectivity.

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(−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor

N. G. Bowery, D. R. Hill & A. L. Hudson
Department of Pharmacology, St Thomas's Hospital Medical School, London SE1, UK
A. Doble*, D. N. Middlemiss, J. Shaw & M. Turnbull
Departments of Biochemistry & Biology, ICI Pharmaceuticals Ltd, Mereside, Alderley Park, Macclesfield, Cheshire, UK

The existence of a receptor for γ-aminobutyric acid (GABA) on neurones of the mammalian central nervous system (CNS) is now firmly established[1–3]. It is generally accepted that bicuculline (and its methohalide salts) is an antagonist of the actions of GABA[4,5], although resistance to bicuculline has been described[6,7]. The view that bicuculline prevents GABA from interacting with a membrane recognition site is supported by results obtained in radiolabelled ligand binding studies[8,9]. Bicuculline-sensitive GABA receptors are not confined to neurones in the CNS but are also present on neurones and axons of the peripheral nervous system[10,11]. Their existence on neurones in sympathetic ganglia made us consider the possibility that they are also present on the terminals of such neurones. This has recently been tested[12,13] by assuming that if GABA depolarises the terminals in a manner similar to the cell bodies, evoked transmitter output might be decreased (see ref. 14). GABA (ED_{50}, 4μM) clearly decreased the evoked release of accumulated ^{3}H-noradrenaline from rat atria *in vitro* and ^{3}H-acetylcholine from preganglionic terminals in the rat superior cervical ganglion *in vitro* without affecting the basal release of tritium. In neither system was the effect of GABA antagonised by bicuculline methobromide, even though the ganglion terminal depolarisation was[13]. This suggested that the two phenomena, depolarisation and inhibition of transmitter release, were separate. The decrease in transmitter release not surprisingly leads to a decrease in the postsynaptic response[15]. Again, the decrease in response was not prevented by bicuculline or other GABA antagonists. We believe that these results indicate the presence of a novel GABA receptor on nerve terminals, a theory supported by results obtained with a variety of GABA analogues. For example, 3-aminopropane sulphonic acid (3-APS) which is at least as active as GABA at bicuculline sensitive sites[10,16,17] is inactive at the terminal receptor. By contrast, the analogue baclofen (β-chlorophenyl GABA) is inactive at bicuculline-sensitive sites[18,19] but is as active as GABA in reducing evoked transmitter output[15]. This effect of baclofen is stereospecific, the (−) isomer being >100-fold more active than the (+) isomer[15]. We now report the presence of the novel GABA receptor within the mammalian CNS.

The technique used in this study was first to allow slices of CNS tissue to accumulate radiolabelled transmitter and then to examine the effect of GABA and its analogues on the basal and K^+-evoked release into fresh superfusion fluid. Three areas of the rat CNS were chosen, the cerebellum, striatum and frontal cortex. Cerebellar slices were incubated in ^{3}H-noradrenaline (13 Ci mmol^{-1}, Amersham), striatal slices in ^{3}H-dopamine (24.8 Ci mmol^{-1}, NEN) and cortical slices in ^{3}H-serotonin (29.2 Ci mmol^{-1}, NEN). The procedures adopted in each case are given in Figs 1 and 2 legends.

The rate coefficient for basal tritium release from the slices was approximately 0.4% min^{-1}. Superfusion of these slices for 2 min with Krebs' solution containing added KCl (final concentration 25 mM) increased the tritium overflow by 100–400% of the basal release. The increase was transient such that the release rate returned to the basal level by the next 4-min collection period. In every experiment the response to K^+ addition declined with repeated applications (Fig. 1*b*). K^+ additions were therefore limited to three in any one experiment. Control experiments indicated that the increase in tritium overflow in each case was Ca^{2+}-dependent. Removal of Ca^{2+}

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* Present address: MRC Neuropharmacology Unit, Hills Road, Cambridge, UK.

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from the superfusion fluid with the addition of EDTA (1 mM) or Mg^{2+} (2.0 mM) prevented K$^+$-induced release.

The effect of GABA and baclofen on K$^+$-evoked release of tritiated noradrenaline from cerebellar slices is shown in Fig. 1. Each histogram bar corresponds to the amount of tritium released by 25 mM KCl plotted as a percentage of the initial response. Each panel shows the mean data from five experiments. Slices from the same animal were used in one of the five experiments in each panel. Figure 1*b* shows the responses to successive additions of KCl. In Fig. 1*a* and *c*, (±)baclofen (100 μM) and GABA (100 μM), respectively, were added 1 min before the second period of superfusion with KCl. In both cases there was ~20% reduction in the expected increase in tritium overflow. When these experiments were repeated with bicuculline methobromide (100 μM) present in the superfusion medium, neither the release evoked by K$^+$ nor the reduction in evoked release produced by GABA and baclofen were affected.

3-APS (100 μM) failed to mimic the action of GABA (three experiments). Pretreatment with this GABA analogue did not reduce the K$^+$-evoked release whereas GABA (100 μM) in the same series of experiments caused the expected reduction. The effect of baclofen was stereospecific; (+)baclofen (100 μM) failed to reduce ^{3}H-noradrenaline release whereas the effect of the (−)isomer was comparable with that of the racemic form.

Similar results were obtained on the evoked release of ^{3}H-dopamine from striatal slices and ^{3}H-serotonin from cortical slices (Fig. 2). The output of both of these transmitter substances was depressed by baclofen and GABA although the response to GABA was variable. In the presence of bicuculline, however, only depressant effects were seen. 3-APS was again without depressant activity on transmitter overflow in both systems.

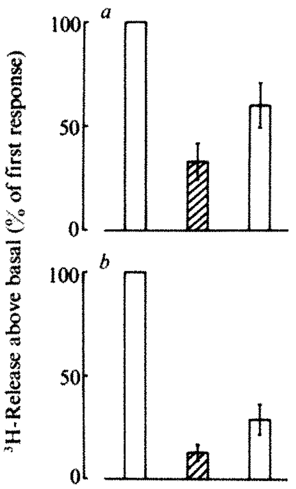

Fig. 2 Depression of K$^+$-evoked release of ^{3}H-dopamine (*a*) and ^{3}H-serotonin (*b*) by (±)baclofen. Ribbons (0.25 mm × 0.25 mm) of striatum (*a*) or frontal cortex (*b*) were obtained from 200-g male rats. The tissue was incubated for 15 min at 37 °C in 5 ml Krebs' solution containing pargyline (1 μM), ascorbic acid (0.2 mM) and either ^{3}H-dopamine (0.1 μM) or ^{3}H-serotonin (0.1 μM). The tissue was then rinsed briefly and aliquots containing approximately 5 mg tissue were transferred to 200-μl superfusion chambers. Superfusion at 0.5 ml min^{-1} was then begun but the collection of 4-min samples was delayed for a further 30 min. The tritium content of each sample was determined by liquid scintillation spectrometry. In each experiment potassium chloride (final concentration 25 mM) was added to the superfusion fluid on three occasions (for 2-min periods at 12-min intervals). The increase in tritium release provoked by each K$^+$ addition and represented by histogram bars has been plotted as a percentage of the initial response. The data in *a* and *b* are mean values ± s.e.m. derived from 16 experiments in each case. (±)Baclofen (100 μM) was present 1 min before and during the second period of K$^+$ addition.

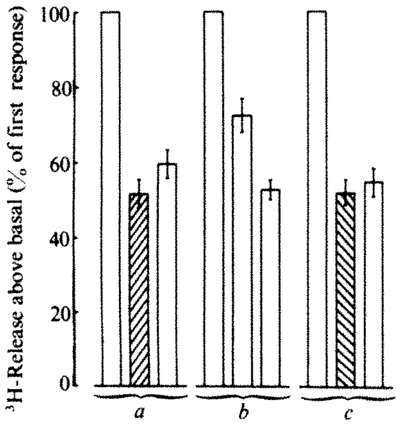

Fig. 1 Effect of GABA and (±)baclofen on the K$^+$-evoked release of ^{3}H-noradrenaline from rat cerebellar cortex slices. Three or four slices of cortex (0.25 mm thick) obtained from decapitated male rats were incubated for 20 min at 32 °C in 1 ml Krebs' solution containing 0.8 μM ^{3}H-noradrenaline, 0.5 mM iproniazid and 0.1 mM ascorbic acid. The slices were then incubated for a further 20 min in radioactive-free solution at 32 °C before transferring to a superfusion chamber. Oxygenated superfusion fluid was delivered at 0.5 ml min^{-1} and 4-min samples collected consecutively. The tritium content of each sample was determined by liquid scintillation spectrometry. Potassium chloride was added (final concentration = 25 mM) to the superfusing fluid for 2-min periods at intervals of 16 min. Each batch of three or four slices was subjected to only three additions of K$^+$. The histogram bars represent the increase above basal of the release of tritium in response to K$^+$ addition. Each has been plotted as a percentage of the initial response. The data are derived from five animals from each of which three batches of slices have been treated separately. Slices from the same animal were used for a control experiment (*b*, each bar corresponding just to added K$^+$), an experiment where (±)baclofen (100 μM) was added 1 min before and during the second period of K$^+$ addition (*a*) and an experiment where GABA (100 μM) was similarly added (*c*). Vertical lines on each bar represent s.e.m.

The possibility that GABA affects neurotransmitter release in the mammalian CNS has been previously considered by many authors. However, conflicting results have been obtained varying from no effect to facilitation of basal or K$^+$-induced release[20–30]. These variations may stem from the differences in tissue dimensions used in individual studies or from differences in the duration of superfusion with high K$^+$ and GABA. A variable which we have considered is the effect of different K$^+$ concentrations. This we have examined on the cerebellar release of ^{3}H-noradrenaline. On increasing the K$^+$ concentration to 35 mM, the reduction in evoked release produced by GABA became more apparent than that at 25 mM. However, at 15 mM K$^+$, GABA (100 μM) potentiated the evoked release (release in the presence of GABA = 113 ± 4.9% of initial response compared with 80 ± 11.0% in control experiments; means ± s.e.m., $n = 6$). Potentiation was never obtained with baclofen. Even in the presence of 15 mM K$^+$ baclofen reduced the evoked release. If the tissue was superfused with bicuculline methobromide (100 μM), the potentiation produced by GABA was reversed such that a reduction in evoked release was again observed.

Baclofen, like GABA, is a powerful neuronal depressant in the mammalian CNS and it has been suggested that this effect of baclofen stems from a suppression of transmitter release as well as from a direct postsynaptic action[18]. However, despite its close structural resemblance to GABA, baclofen is generally not considered to be a GABA receptor agonist because of its insensitivity to GABA antagonists[19,31] (but see ref. 18) and inability to displace ^{3}H-GABA from receptor binding sites[32,33] (but see ref. 34). Our findings suggest that baclofen may mimic the action of GABA at a novel site on nerve terminals. Despite failing to obtain an antagonist for this receptor, our observations[15] of cross-desensitisation, lack of additivity with supramaximal doses and parallelism of log dose–response curves in peripheral models indicate that baclofen and GABA do act at the same site. Potashner[35] and Nistri[36] have previously shown that the evoked release of ^{3}H-glutamate and acetylcholine from guinea pig cortical slices and from hemisected spinal cord, respectively, is reduced by baclofen. It is tempting to believe that their observations result from an action at the same bicuculline-insensitive GABA receptor.

In conclusion, we have shown the existence of a novel GABA receptor which, when activated, decreases transmitter output. As (−)baclofen is the active isomer not only at this receptor[15] but also in producing neuronal depression[37], its mechanism of action in the therapy of spasticity may indeed be linked with GABA.

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Compensatory bilateral changes in dopamine turnover after striatal kainate lesion

Kurt Andersson, Robert Schwarcz & Kjell Fuxe
Department of Histology, Karolinska Institute, Stockholm, Sweden

Diseases of the extrapyramidal systems like Parkinson's disease and Huntington's chorea have been closely associated with the basal ganglia[1,2]. In particular, the prominent symmetrical nigrostriatal dopaminergic systems play an important role in pathogenesis and symptomatology of motor disorders[3–5]. Biochemical[6,7] and electrophysiological[8,9] data indicate a functional relationship between the nigrostriatal pathways on the two brain hemispheres. Unilateral pharmacological manipulations result in short-term changes in contralateral dopamine (DA) release, and surgical lesions on one side lead to long-term alterations in the firing rate of contralateral dopaminergic neurones. We report here the effects of unilateral kainic acid-induced lesions of striatal cell bodies on ipsi- and contralateral striatal DA turnover. We present evidence that morphologically intact dopaminergic neurones on both sides possess compensatory mechanisms which can counteract striatal synaptic asymmetries. Such mechanisms could be responsible at least in part for the maintenance of symmetrical motor behaviour in unilaterally lesioned animals.

After anaesthesia with Nembutal (60 mg per kg, intraperitoneally (i.p.)), male Sprague–Dawley rats (200 g) were positioned in a David Kopf small animal stereotaxic apparatus and kainic acid (1 μg in 0.5 μl phosphate-buffered saline (PBS), *p*H 7.4) was infused unilaterally into the neostriatum (coordinates: 7.9A; 2.6L; 4.8V) as described previously[10]. The animals were killed 2 days to 2 months later by decapitation and the brains rapidly processed according to the Falck–Hillarp technique for the cellular demonstration of catecholamines[11]. The DA stores were measured microfluorimetrically[12]. DA turnover was determined by following DA disappearance after tyrosine hydroxylase inhibition[13]. For this purpose endogenous DA was measured at zero time and at 1 and 2 h after the administration of α-methyltyrosine methyl ester (H44/68, 250 mg per kg, i.p.). Analysis was performed on injected (kainic acid or PBS), contralateral or intact control striata. The medial parts of the caudate nucleus were assessed individually as described previously[14]. To control for the lesion procedure three kainate-injected rats were killed at 2, 10, 35 and 60 days respectively after the lesion, and their brain processed for morphological analysis by light microscopy.

As confirmed in this study, striatal injections of 1 μg kainic acid induce marked degeneration of neuronal cell bodies throughout the nucleus caudatus, nucleus accumbens and piriform cortex and in parts of the globus pallidus and the part of the neocortex overlying the injected striatum. Also, in accordance with recent immunohistochemical and histological findings, dopaminergic nerve terminals appear to be spared by the lesion with the exception of a small area of nonspecific damage around the needle track[14,15]. No morphological changes could be detected on the contralateral striatum.

Two days after kainic acid injection, DA levels and DA turnover in the medial part of the caudate nucleus were

Table 1 Effects of striatal kainic acid injection on DA levels in medial part of caudate nucleus

Days after treatment	Ipsilateral (%)	Contralateral (%)	Normals (%)
	Kainic acid injected		
2	130 ± 8*(4)	110 ± 6(4)	100 ± 7(4)
10	118 ± 9	116 ± 9	100 ± 3
35	146 ± 12**†	106 ± 4	100 ± 3†(4)
	PBS injected		
35	123 ± 4	106 ± 2	100 ± 3(5)
	Kainic acid injected		
60	—	124 ± 4**	100 ± 4

Kainic acid (1 μg dissolved in 0.5 μl saline) was injected unilaterally in normal male rats 2, 10, 35 or 60 days before killing. PBS (0.5 μl) was injected unilaterally after 35 days. Normal male rats served as controls. Animals were killed by decapitation and the caudate nucleus was taken for histochemical analysis of the DA levels[13]. In the turnover experiments H44/68 (250 mg per kg, i.p.) was injected 1 h or 2 h before decapitation[12]. In some cases at the 35 day time point the entire ipsilateral caudate was taken for biochemical analysis. DA levels are expressed as % of control animals, means ± s.e.m. In the biochemical analysis 100% DA = 8,291 ± 279 ng per mg tissue. The fluorescence values obtained in the quantitative microfluorimetrical analysis are given in arbitrary fluorescence units. $n = 6$ if not otherwise noted within parentheses. Statistical analysis according to Mann–Whitney U-test (60 day point) or Wilcoxon: one-way classification, treatment versus control (2, 10 and 35 days treatment). * $P < 0.05$; ** $P < 0.01$. The measurements were made at around level A8500 according to König and Klippel[29].

† Biochemical analysis performed on the whole ipsilateral caudate after killing at 35 days.

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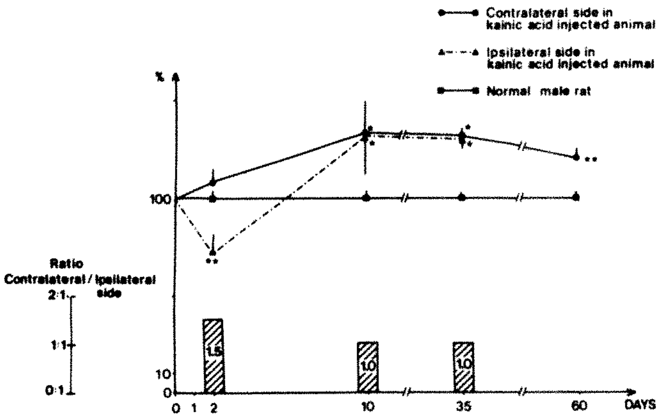

Fig. 1 DA turnover changes in ipsi- and contralateral striata at various times after kainate injection. Data represent values in the medial part of the caudate nucleus 2 h after H44/68 treatment. Values below 100% indicate increased DA turnover, and vice versa. Original data are listed in Tables 1 and 2. Vertical bars indicate the ratios of H44/68-induced DA disappearance between contra- and ipsilateral striata. Statistical analysis according to Wilcoxon: one-way classification, treatment versus control. ★ $P<0.05$; ★★ $P<0.01$.

increased significantly on the injected side as compared to contralateral or control values (Tables 1 and 2). This increase, synchronous with increased tyrosine hydroxylase activity, increased L-dopa accumulation after dopa-decarboxylase inhibition[10,14] and contralateral turning of the lesioned animal[10], seems to reflect a functional response of the dopaminergic nerve terminal to an indirect intrastriatal DA release caused by kainic acid[16] and/or to the loss of inhibitory striatonigral feedback mechanisms after degeneration of striatal neurones[17,18].

By 10 days after the lesion, the increase in DA levels is not as large as 2 days post-injection or has disappeared (Table 1). The DA turnover is now reduced on the lesioned and contralateral side when compared to uninjected control striata (Table 2). The decrements in DA turnover also extend to the central part of the caudate nucleus (level A 8500 according to König and Klippel[29]; L: 2.0 and V extending from +2.0 to 0.0) and to other brain areas rich in dopaminergic nerve terminals like the nucleus accumbens and to a lesser degree the tuberculum olfactorium (data not shown). At 35 days post-lesion significant increases of DA levels were found in the caudatus of the ipsi- and contralateral side; at 60 days significant increases in DA levels were observed in the medial caudate. DA turnover decreases on the contralateral side remain significant at 35 and 60 days after the lesion. At 35 days the biochemical analysis of the entire ipsilateral caudatus still showed reduced DA turnover. Note that injections of PBS-saline into the caudate nucleus did not produce any changes in DA levels and turnover as illustrated in Table 2 for the 35-day post-lesion time point.

At 2 to 3 days after the lesion animals change the direction of spontaneous rotation and start turning towards the lesioned side[10]. Because of the concomitant general hypoactivity of the animals no quantitative evaluation of this turning behaviour (less than one full turn in 5 min) has been performed to date. However, the ipsilateral preference at this stage can be easily observed and the circling strongly accentuated by the administration of dopaminergic drugs[19]. At 5 to 8 days after the lesion the rats stop rotating spontaneously. Figure 1 shows the DA turnover changes at various times after a unilateral striatal kainic acid lesion. Turnover ratios between injected and contralateral striata approach 1.0 at a time when motor asymmetry subsides. Symmetry in striatal DA turnover may therefore contribute to motor symmetry and represent a valid neurochemical marker for the degree of symmetry of motor behaviour in the rat.

The anatomical pathways and biochemical relay systems by which the relevant information is transferred from the lesioned to the contralateral hemisphere are a matter for speculation. Connections within the basal ganglia and bilateral projections from the motor cortex to the caudate nucleus may provide the brain with a network of cross-linkages by which fluctuations in one nigrostriatal system may trigger alterations in contralateral DA turnover[20,21]. However, little is known about the biochemical mechanisms associated with this information flow. Dendritic release of DA in the substantia nigra[22–24] may be involved in this process. It has been shown recently that unilateral nigral DA release can inhibit dendritic DA release in the contralateral substantia nigra while simultaneously increasing contralateral striatal DA release[6].

Table 2 Effects of striatal kainic acid injection on DA turnover in medial part of caudate nucleus

Days after treatment			Ipsilateral (%)	Contralateral (%)	Normals (%)
			Kainic acid injected		
		0 h	100±6 (4)	100±6 (4)	100±7 (4)
2	H44/68	1 h	54±4** (5)	77±7 (5)	76±3 (5)
	H44/68	2 h	42±6**	63±4	58±3
		0 h	100±8†	100±8†	100±3
10	H44/68	1 h	92±9*† (5)	80±10† (5)	76±3 (5)
	H44/68	2 h	77±11*†	78±12*†	58±3
		0 h	100±8	100±4	100±3 (4)
35	H44/68	1 h	71±4 (5)	80±3 (5)	62±3 (4)
	H44/68	2 h	64±3* (4)	78±2* (5)	49±2 (4)
			PBS injected		
		0 h	100±3†	100±2	100±3 (5)
35	H44/68	1 h	75±5†	75±4	72±2 (4)
	H44/68	2 h	49±4 (4)	57±2 (5)	59±3 (3)
			Kainic acid injected		
		0 h	—	100±3	100±4
60	H44/68	1 h	—	87±4	79±4
	H44/68	2 h	—	77±3**	64±3

Experiments were performed as detailed in Table 1. Values for DA turnover are given as % of levels at zero time. Statistical comparisons were always made with the respective normal group. * $P<0.05$; ** $P<0.01$.

† Biochemical analysis performed on the whole ipsilateral caudate after killing at 35 days.

Circling in the rat has commonly been associated with dopaminergic receptor mechanisms[25]. Striatal postsynaptic DA-sensitive adenylate cyclase activity and dopaminergic haloperidol or spiperone binding sites decrease by 85% and 40% respectively, by 2 to 6 days after striatal kainate lesions[26,27]. Receptor loss, which can be demonstrated up to 12 months after kainic acid lesions[28], is due to a reduction in the number of binding sites rather than to affinity changes and no alterations can be demonstrated on the contralateral side. Postsynaptic parameters therefore do not seem to contribute significantly to the cessation of spontaneous turning behaviour at 5 to 8 days after the lesion. In contrast, our present data indicate that rotation in the rat can be regulated at the presynaptic level. It seems likely that compensatory changes in striatal DA turnover represent one of the mechanisms by which rats can maintain symmetrical postures in spite of a drastic unilateral striatal impairment. Clearly this mechanism can only partly explain the return of symmetry, since DA receptor loss exists on the operated side and DA release still occurs on the contralateral side. However, it is conceivable that close scrutiny of origin and regulation of this endogenous adaptational process may contribute significantly to our understanding of human movement disorders.

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Selective conversion of β-endorphin into peptides related to γ- and α-endorphin

J. P. H. Burbach, J. G. Loeber, J. Verhoef, V. M. Wiegant, E. R. de Kloet & D. de Wied

Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands

β-Endorphin (β-LPH$_{61-91}$) is a well known endogenous opioid ligand. It and related peptides have recently been implicated in the control of adaptive behaviour. Smaller β-endorphin fragments appeared to be more active moieties than the parent molecule in a number of behavioural situations[1]. Their effects seemed to occur independently of interaction with opiate receptor sites in the brain. Moreover, elimination of the opiate-like properties of γ-endorphin (β-LPH$_{61-77}$) by removing the N-terminal amino acid yielded des-tyrosine-γ-endorphin (β-LPH$_{62-77}$, dTγE) which had greater behavioural activity than γ-endorphin[2,3]. The CNS effects of dTγE resembled those of neuroleptic drugs in several test systems. α-Endorphin (β-LPH$_{61-76}$) exerted effects opposite to those of dTγE and in some aspects its activity was comparable to that of psychostimulant drugs[4]. This opposition of effects suggests that a balance between γ- and α-type endorphins is involved in the control of brain function[5]. We report here that either γ-endorphin and dTγE or α-endorphin and des-tyrosine-α-endorphin (β-LPH$_{62-76}$, dTαE) can be formed preferentially from β-endorphin by enzymes associated with an enriched synaptosomal plasma membrane fraction from brain. It is suggested that these enzymes have a role in brain homeostatic mechanisms by regulating the generation of these substances.

Synaptosomal plasma membrane (SPM) preparations from rat brain were obtained by differential centrifugation (see legend to Fig. 1). β-Endorphin (20 nmol) was incubated with the SPM preparation (3.5 mg protein) at 37 °C for 30 min in 1 ml saline buffered with either 25 mM sodium phosphate (*p*H 7.4, 6.7 and 5.9) or 25 mM sodium acetate (*p*H 5.0). The reaction was stopped by heating the suspension at 95 °C for 10 min and the membranes were removed by centrifugation. The supernatant was subjected to high-pressure liquid chromatography (HPLC) fractionation and the peptide-containing fractions were collected. The products were identified by combined application of HPLC and radioimmunoassay (RIA). The HPLC system was able to separate very similar β-endorphin fragments in a highly reproducible manner (Fig. 1). Analysis of their immunoreactivity provided an additional criterion for the identity of peptides cross-reacting in the respective RIA systems.

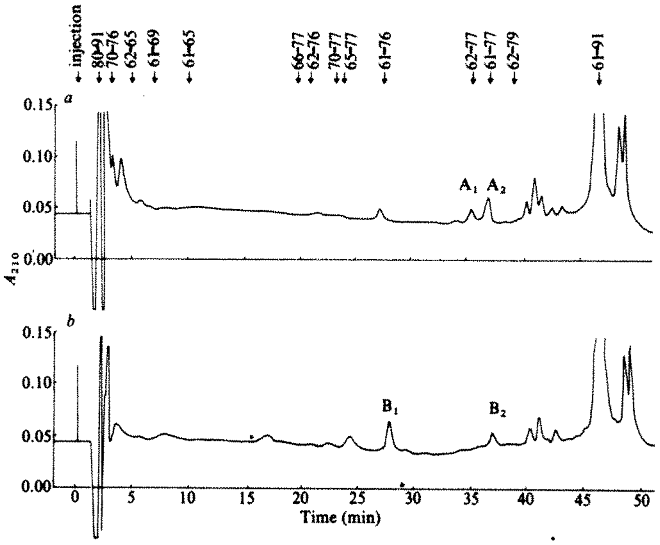

Fig. 1 HPLC fractionation of peptides formed by conversion of β-endorphin by enzymes associated with a SPM preparation. A 10% (w/v) homogenate of rat forebrain in 0.32 M sucrose was centrifuged at 1,000g_{av} for 10 min. The supernatant was centrifuged at 10,000g_{av} for 20 min and the resulting crude mitochondrial–synaptosomal fraction was lysed in distilled water (5 ml per g tissue) at 0 °C for 30 min. The supernatant obtained by centrifugation of the suspension at 10,000g_{av} for 20 min contained the synaptosomal plasma membranes. The membranes were washed and stored at −20 °C. Incubations were carried out as described in the text. Samples (700–800 μl) were subjected to HPLC fractionation on a μBondapak C_{18} reversed-phase column (0.39×30 cm) eluted with a concave gradient of 10 mM ammonium acetate, *p*H 4.15 (X) and methanol, containing 1.5 ml l^{-1} acetic acid (Y). Initial conditions: $X:Y=70:30$, final conditions: $X:Y=25:75$. The flow rate was 2 ml min^{-1}. HPLC chromatograms of peptide mixtures were obtained after incubation of β-endorphin at *a*, *p*H 6.7 and *b*, *p*H 5.9. The retention times of a series of reference peptides are indicated at the top. Fractions A_1 and A_2 contained dTγE and γ-endorphin respectively; fractions B_1 and B_2 contained α-endorphin and γ-endorphin respectively.

Briefly, the specificities of the antisera were as follows. For the anti-α-endorphin serum, the percentage cross-reaction compared to α-endorphin was 110% for dTαE, 2% for γ-endorphin, 5% for dTγE, 1% for β-endorphin and 0.13% for methionine enkephalin. For the anti-γ-endorphin serum, dTγE cross-reacted 120%, α-endorphin 8%, dTαE 0.6%, β-endorphin 4% and methionine enkaphalin 0.02%. Details of the RIA systems have been given elsewhere[6].

β-Endorphin was digested by enzymes present in the SPM preparation. HPLC fractionation of the digests revealed the presence of β-endorphin fragments with retention times identical to those of dTγE, γ-endorphin and α-endorphin in the HPLC system (Fig. 1). Their identity was confirmed by analysis of the immunoreactivity in the appropriate RIA systems (Fig. 2). Essentially the same results were obtained when β-endorphin was incubated with a purified SPM preparation obtained by sucrose density centrifugation as described by Terenius[7].

RIA of β-endorphin fragments collected during HPLC fractionation of the digests enabled us to quantitate α-endorphin, dTαE, γ-endorphin and dTγE. The accumulation of fragments was highly *p*H-dependent. The largest amounts of α-endorphin

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and small amounts of γ-endorphin and dTγE were detected in fractions obtained at *p*H 5.9. The same *p*H seemed to be optimal for the accumulation of dTαE, a minor component of the digests. Increasing the *p*H to 6.7 completely reversed the ratio of α- to γ-type fragments: α-endorphin decreased whereas there was a marked increase in both γ-endorphin and dTγE (Fig. 3).

Austen and Smyth, using β-endorphin with the N-terminal tyrosine residue labelled with ^{125}I have demonstrated the production of γ-endorphin at *p*H 7.4 and of α-endorphin at *p*H 5.0 by brain membranes; most of the methionine-enkephalin was also found at *p*H 5.0 (refs 8, 9). Their experiments were performed in the presence of bacitracin so as to protect the peptide against release of the labelled amino acid residue. The present studies were designed to detect the formation of des-tyrosine endorphins and to extend previous observations to synaptosomal plasma membranes.

Peptide fragments accumulate as a result of both formation and degradation. It has been suggested that initial steps in the degradation of β-endorphin by brain extracts involves cleavages by several endopeptidases, followed by rapid breakdown of the resulting intermediates to free amino acids[10,11]. The present results show that an enriched synaptosomal plasma membrane preparation contains an enzyme system for the conversion of β-endorphin into γ-endorphin, dTγE, α-endorphin and dTαE. Either dTγE and γ-endorphin or α-endorphin accumulated preferentially depending on the *p*H. Thus, the *p*H appears to determine whether the breakdown of γ-endorphin and dTγE or that of α-endorphin is the rate-limiting step in the enzymatic conversion of β-endorphin. As a consequence of the mechanism of degradation rather low amounts of the accumulated peptides were detected.

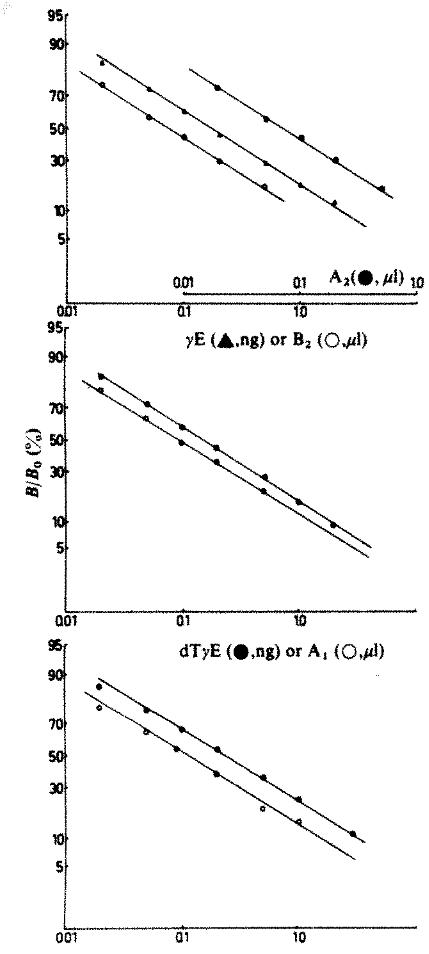

Fig. 2 Immunological activity of peptides in fractions A_1, A_2, B_1 and B_2 (see Fig. 1 legend) as tested in radioimmunoassay systems for dTγE, γ-endorphin, α-endorphin and γ-endorphin, respectively. Fractions of the HPLC eluate containing the β-endorphin fragments were collected and methanol was evaporated *in vacuo* at 60 °C. Aliquots of the residual solution (indicated as μl) were subjected to the appropriate RIA. Ordinate shows final fraction bound as a percentage of initial bound.

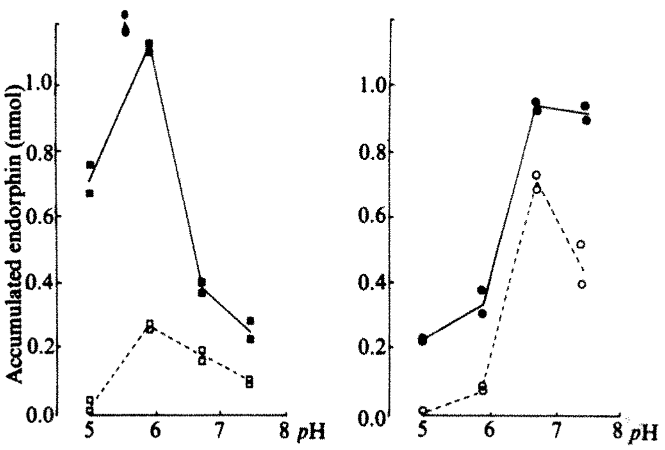

Fig. 3 *p*H dependence of the accumulation of α-endorphin (■), dTαE (□), γ-endorphin (●) and dTγE (○) upon incubation of β-endorphin with a SPM preparation. Digests were prepared and fractionated as described in the text and in the legend to Fig. 1. Fractions containing the individual β-endorphin fragments were collected during HPLC fractionation and methanol was removed *in vacuo* at 60 °C. Peptides contained in the residual solution were quantitated in the RIA systems for the various β-endorphin fragments. Values were obtained from two separate experiments.

Recently, using HPLC and RIA we have detected des-tyrosine-endorphins in biological material in addition to α-, γ- and β-endorphin. dTγE and dTαE are present in rat pituitary[6], in rat brain and in human cerebrospinal fluid (J. P. H. B. *et al.*, in preparation). Together with the present study this observation supports our view that des-tyrosine-endorphins are endogenous peptides and are formed by selective cleavage of β-endorphin.

β-Endorphin fragments may be factors involved in behavioural adaption. According to the hypothesis put forward by De Wied[5], normal brain functioning would require a balance between dTγE or a peptide related to it and α-type endorphins. Consequently, the enzyme system acting in our experiments may provide a regulatory mechanism for the turnover of the two ligands. The preferential formation of dTγE and γ-endorphin or α-endorphin over a narrow, near physiological *p*H range shows that the enzyme system can be modulated. The experimental *p*H changes may have mimicked physiological processes leading to this modulation. Thus, by showing the formation of dTγE, γ-endorphin and α-endorphin by enzymes present in an enriched synaptosomal plasma membrane fraction, the experiments described here may have provided a biochemical basis for the hormonal regulation of adaptive behaviour.

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Parallel pathways for transduction of chemotactic signals in *Escherichia coli*

Gerald L. Hazelbauer & Peter Engström

The Membrane Group, The Wallenberg Laboratory,
University of Uppsala, S-752 37 Uppsala, Sweden

Bacteria respond transiently to tactic stimuli[1], which indicates that cells adapt to new levels of stimulation. Receptors[2,3] send signals to a tumble regulator that controls the balance between directions of flagellar rotation[4], creating a pattern of swimming and tumbling that results in taxis[5]. In *Escherichia coli*, mutations in *tsr* and *tar* affect receptor–regulator linkages, eliminating responses mediated by two different groups of receptors[6–9]. Sensitivity to most sugars is not reduced by either mutation but observations that *tsr–tar* double mutants do not respond to spatial gradients of any compound led to the suggestion that the *tsr* and *tar* products together provide a necessary final step in transduction from all receptors[7,8]. Another class of pleiotropic taxis mutations, *trg*[10–13], causes defective transduction from only two receptors, those for galactose and ribose. We were therefore interested in determining whether the *trg* function represented an early stage of transduction or whether it was analogous to the *tsr* and *tar* functions. We have examined the sensitivity of *tsr* and *tar* double mutants to temporal gradients, prompted by a report that such gradients of galactose or ribose evoke responses in those strains[14]. We report here that *tsr–tar* double mutants were sensitive to stimulation by compounds to which both single mutants respond but were drastically defective in adaptation to these stimuli. We suggest there are at least four parallel, single-component pathways for transduction of tactic signals, from the Tsr, Tar, Trg and enzyme II groups of receptors.

We used four *tsr–tar* double mutants (see Table 1 legend) and confirmed that they tumble very infrequently[7–9]. *tsr–tar* cells tethered to a glass surface spun only in the counterclockwise, smooth-swimming direction, in contrast to wild-type cells which frequently reverse their direction of rotation. However, undisturbed cells initiated reversals at times ranging from 5 to 40 min after tethering and subsequently behaved much like wild-type cells. We believe the delayed onset of a normal rotational pattern results from the adaptational defect of these mutants documented below. Lack of tumbling by swimming cells and absence of clockwise rotation by tethered cells would then be a reflection of extended times necesseary for adaptation to stimuli received during prior manipulation (such as changes in temperature or concentration of O_2 or K^+ ion).

After tethered *tsr-tar* cells had begun to exhibit normal rotational patterns, we applied maximal temporal stimuli, gradients from 0 to 10 mM, of the compounds listed in Table 1. Compounds inactive in either *tsr* or *tar* mutants were predictably inactive in the double mutants. Two amino acids, asparagine and cysteine, which evoke significant responses in both *tsr* and *tar* mutants[6], did not stimulate a double mutant. This is consistent with the notion that receptors for these compounds interact with both the *tsr* and *tar* transductional pathways and one or the other must be functional for a response[7,8]. In contrast, *tsr–tar* double mutants responded to sugars in the same way as wild-type cells, by complete suppression of tumble-mode rotation. However, adaptation was significantly delayed after maximal stimuli of sugars recognised by enzyme II receptors[3,15] and did not occur even after prolonged times for those recognised by *trg*-related receptors. Delayed adaptation did occur after moderate stimuli of allose and fucose, non-metabolisable analogues of ribose and galactose, respectively (Fig. 1). Behaviour of a *tsr–tar* double mutant lacking the galactose receptor as the result of an *mglB* mutation[16] (Table 1) demonstrates that responses observed in *tsr–tar* double mutants require the appropriate chemoreceptor. In addition, it eliminates the possibility that responses to enzyme II sugars are caused solely by low level contamination with galactose or glucose, which are recognised at micromolar concentrations by the galactose receptor.

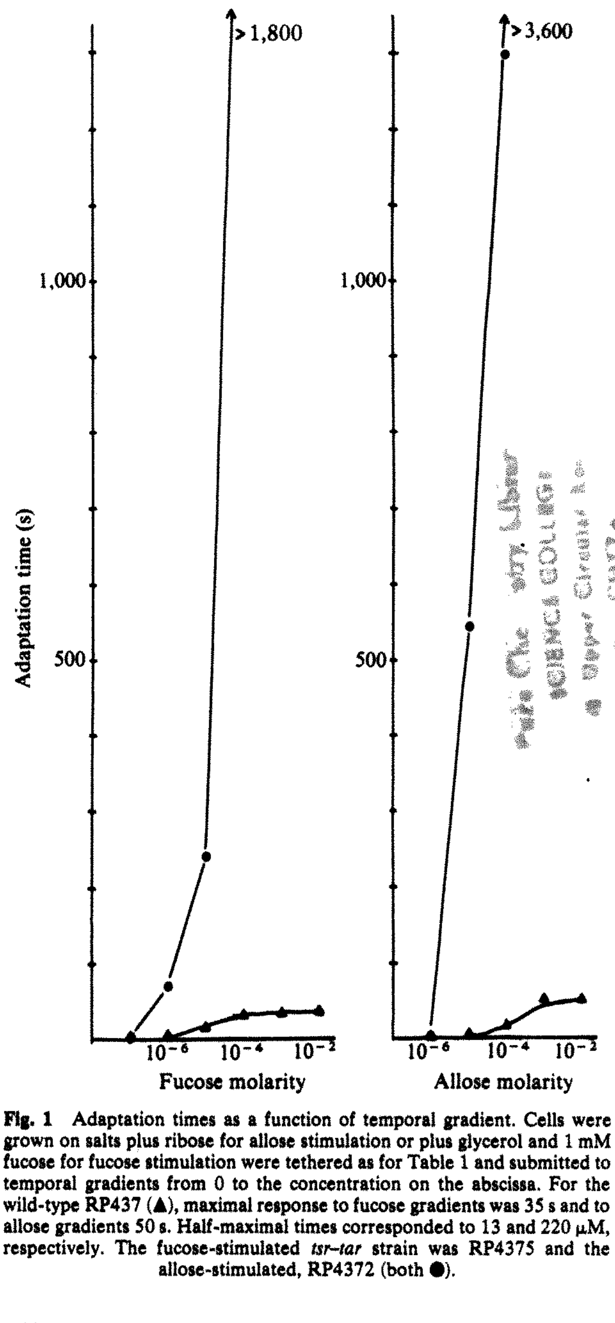

Fig. 1 Adaptation times as a function of temporal gradient. Cells were grown on salts plus ribose for allose stimulation or plus glycerol and 1 mM fucose for fucose stimulation were tethered as for Table 1 and submitted to temporal gradients from 0 to the concentration on the abscissa. For the wild-type RP437 (▲), maximal response to fucose gradients was 35 s and to allose gradients 50 s. Half-maximal times corresponded to 13 and 220 μM, respectively. The fucose-stimulated *tsr–tar* strain was RP4375 and the allose-stimulated, RP4372 (both ●).

When an attractant recognised by an enzyme II receptor was washed away from adapted *tsr–tar* cells, reversals between clockwise and counterclockwise rotation ensued at high frequency for periods of the order of a minute. Wild-type cells stimulated by 10 mM to 0 gradients of those sugars exhibit only a few seconds of clockwise rotation. Thus, it seems that *tsr–tar* double mutants are also sensitive to unfavourable stimuli mediated by sugar receptors and, as in the case of favourable stimuli, are defective in adaptation.

A *trg* null mutation[12] was introduced into the four *tsr–tar* double mutants. We found that tethered cells of *tsr–tar–trg* triple mutants rotated exclusively in the smooth-swimming direction and, in contrast to the double mutants, never showed a relaxation to normal alternating rotational behaviour. We believe this phenotype to be a more drastic extension of the adaptational defect of the double mutants, implying that the *trg* product is involved in adaptation as well as transduction.

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Table 1 Adaptation times of tethered cells to maximal temporal stimuli

Attractant	Receptor	Transducer	Adaptation time (s) WT	WT *mgl*B	*tsr tar*	*tsr tar mgl*B	Ratio *tsr tar*/WT
Galactose	GBP	Trg	30	ND	>1,800	ND	>60
Fucose	GBP	Trg	33	0	>1,800	9	>55
Ribose	RBP	Trg	50	ND	>1,800	ND	>36
Allose	RBP	Trg	48	50	>1,800	>1,800	>37
Glucose*	enz $\text{II}^{\text{glu}*}$	Unident.	ND	52	>1,800	157	3
Fructose	enz II^{fru}	Unident.	41	32	540	390	12
Sorbitol	enz II^{sbl}	Unident.	55	15	642	353	24
N-acetylglucosamine	enz II^{NAG}	Unident.	72	55	480	239	4
Asparate	Unident.	Tar	258	ND	60	0	0
α-methylaspartate	Unident.	Tar	221	ND	26	0	0
Serine	Unident. (2)	Tsr + Tar	790	720	49	18	0.02
Asparagine	Unident.	Tsr + Tar	ND	258	ND	0	0
Cysteine	Unident.	Tsr + Tar	ND	330	ND	0	0

Cells were grown at 37 °C in minimal salts[10] plus ribose (*mgl*B) or ribose and 1 mM fucose (*mgl*B^+). No special measures were taken to induce maximal levels of enzymes II. Flagella were sheared, cells tethered by antiflagella serum, stimulated by temporal gradients at 37 °C and observed on videotape recordings as described elsewhere[11]. Adaptation time for a given cell was the time after stimulation by an attractant when the first complete clockwise rotation occurred. Values given are mean times for 10–20 cells in one or usually two experiments. Standard deviations were 20–30% for short (~60 s) times and somewhat less for longer times. *tsr–tar* double mutants often exhibited poor motility and thus could be tethered without shearing. Such cells initiated normal rotational behaviour (see text) sooner than sheared cells. A change of buffer surrounding tethered cells results in an inhibition of clockwise rotation for an average of 5–10 s in wild-type cells and 30–150 s in *tsr–tar* cells, the values varying for different preparations of tethered cells. These background responses have been subtracted from the raw data to yield the values given. As in the case of *che*X mutants[19], the double mutants had a tendency to lose the ability to rotate clockwise after extended times (> 1 h) in chemotaxis buffer. The wild-type (WT) strain was RP437 (ref. 21) and the *tsr–tar* mutant RP4375 (*tsr* 2·3·4 *tar* 52Δ1). Similar behaviour was observed for RP4372 (*tsr* 518 *tar* 52Δ1), RP4373 (*tsr* 518 *tar* 1) and RP 4376 (*tsr* 2·3·4 *tar* 1). An *mgl*B::Tn*5* mutation (S. Harayama and G.L.H., unpublished) was introduced by transduction, and the absence of the gene product confirmed by SDS-polyacrylamide gel electrophoresis[22]. *trg*2::Tn*10* (ref. 12) was introduced into each *tsr–tar* double mutant by transduction and the presence of the mutation confirmed by transduction of the Tc^r, Trg character back into a wild-type strain. All compounds were obtained from commercial sources and used without further purification. Ratios of mutant to WT response were calculated using values for *mgl*B derivatives where adaptation times of those derivatives were shorter than the parent strain, indicating that the sugar might contain contaminating glucose or galactose. GBP, galactose–glucose-binding protein; RBP, ribose-binding protein; MBP, maltose-binding protein; Unident., unidentified; ND, not determined.

* Glucose is recognised by three receptors, GBP, enz II^{glu} and enz II^{man}. There is very little enz II^{man} receptor activity in these strains.

The relationship between transduction and adaptation merits further discussion. The *tsr* and *tar* products, phenotypically identified as transducers, are directly involved in adaptation to the stimuli they transduce[14]. Adapatation is correlated with carboxylmethylation or demethylation of the two products, termed methyl-accepting protein (MCP) I and II, respectively[7,8]. Recently, an independent MCP III was shown to be methylated on stimulation by compounds recognised by galactose and ribose receptors[13]. MCP III is not methylated in *trg* mutants and may well be the *trg* product. These observations correlate well with our conclusions that *trg* represents an independent transducer that is also involved in adaptation.

tsr–tar double mutants lack only two transducer/MCPs and exhibit correspondingly limited defects in tactic sensitivity. However, the mutants are generally defective in adaptation. Interestingly, *tsr* and *tar* single mutants swim smoothly for longer times than wild-type cells after temporal stimulation by active compounds[7]. The extended times were considered to be enhanced responses[7]; we suggest instead that they indicate moderately defective adaptation. Modest extensions of adaptation times may result in increased accumulations in spatial gradients[6,7], but mutants exhibiting very long adaptation times do not accumulate at all (ref. 7 and the present study). It is striking that *tsr–tar* mutants are as defective in adaptation to maximal stimuli of *trg*-related compounds as are cells unable to methylate because of methionine starvation[17] or a defective methyltransferase[18,19], even though methionine, the transferase and the appropriate transducer/MCP are present.

The extended adaptation times of *tsr–tar* double mutants pose the question of whether methylation levels are correspondingly increased, as might be expected from the correlation of methylation increases and adaptation in wild-type cells[20]. This does not seem to be the case. Double mutants stimulated by gradients of fucose (Fig. 2) or allose (data not shown) exhibit very little detectable methylation, whether the stimulus is one to which the mutant adapts after extended times or one to which it does not adapt in the time of the experiment (see Fig. 1). Basal levels of MCP III methylation in *tsr–tar* mutants are low[13] and adaptation by wild-type strains to maximal *trg*-transduced stimuli corresponds to a 50% increase in MCP III methylation. Thus, it is not possible to conclude from Fig. 2 whether both adapatation and MCP III methylation simply proceed slowly in *tsr–tar* mutants or whether adaptation occurs slowly by a process independent of methylation.

The observation that *tsr–tar* double mutants respond to temporal gradients of sugars demonstrates that the *tsr* and *tar* products together do not transduce signals from all receptors[7–9]. Identification of defective adaptation rather than defective response as the basis for the inability of double mutants to accumulate in spatial gradients of sugars removes the remaining

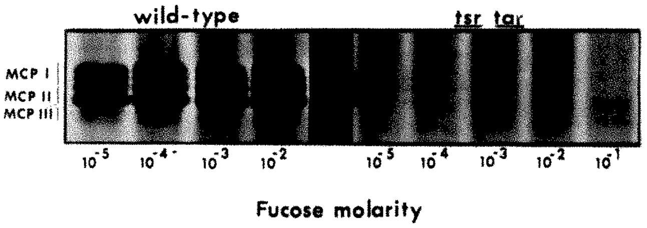

Fig. 2 Methylation patterns of wild-type cells and a *tsr–tar* mutant after stimulation by fucose. Cells were grown in salts plus ribose and 1 mM fucose, washed, resuspended, exposed to [*Me*-^{3}H] methionine (12 Ci mmol^{-1}, Amersham), subjected to temporal gradients from 0 to the concentration indicated and collected 30 min after stimulation essentially as described previously[7]. Approximately 10^8 cells were subjected to electrophoresis on an SDS-10% polyacrylamide gel. The gel was prepared for fluorography and exposed to X-ray film[23]. Only the section of the autoradiogram including the area of known MCPs (apparent molecular weights 56,000–65,000 (ref. 7)) is shown. Wild-type and *tsr–tar* are as in Table 1. The range of methylated bands that constitute MCPs I and II are indicated based on the scheme in ref. 7. We have observed that MCP III includes at least two methylated bands (P.E. and G.L.H., in preparation).

barrier to postulating that the *tsr*, *tar* and *trg* products function as analogous, independent transducers. All data now suggest that the link between any receptor and the tumble regulator is a single transducer protein and that the protein is an MCP, methylated during adapatation to the stimuli it transduces. No observations remain that require postulation of multi-step linkages between receptor and regulator[1,3,13,14].

We suggest that there is at least one additional transducer/MCP, serving enzyme II receptors, as *tsr*, *tar* or *trg* mutations do not eliminate responses to enzyme II sugars[9,11,13]. Also, *trg*-transduced stimuli can be distinguished from enzyme II-mediated stimuli by the inability of *tsr–tar* double mutants to adapt to maximal levels of *trg* stimuli (Table 1).

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Translational control of proinsulin synthesis by glucose

Nobuyuki Itoh & Hiroshi Okamoto*

Department of Biochemistry, Toyama Medical and Pharmaceutical University, School of Medicine, Toyama 930-01, Toyama, Japan

Glucose is known to stimulate proinsulin synthesis in pancreatic islets of rats as well as of many other animals[1], but the mechanism involved remains uncertain. If proinsulin induction by glucose is regulated at the transcriptional level, the amount of proinsulin mRNA may be increased by the administration of glucose. Alternatively, if proinsulin induction is regulated at the translational level, the rate of proinsulin synthesis may not be correlated with the amount of proinsulin mRNA. We have now used a proinsulin cDNA hybridisation method to determine the amount of proinsulin mRNA and the subcellular distribution of proinsulin mRNA in rat pancreatic islets. The results suggest that glucose-induced proinsulin synthesis is mainly achieved by enhancing the translation efficiency of proinsulin mRNA pre-existent on the membrane-bound polysome in pancreatic islets.

Proinsulin mRNA was purified from rat B-cell tumours induced with streptozotocin and nicotinamide, and proinsulin cDNA was synthesised using purified proinsulin mRNA as a template[2–4]. The fidelity of the hybridisation reaction with proinsulin mRNA and its cDNA was assessed from the following evidence: (1) the hybridisation occurred within two orders of magnitude, as expected of a pseudo-first order reaction[3]; (2) the experimental $R_0t_{1/2}$ value (2.1×10^{-4}) was in good agreement with the expected value (2.7×10^{-4}) calculated from the $R_0t_{1/2}$ value of the hybridisation with mouse globin mRNA and its cDNA[3]; (3) a sharp thermal denaturation curve of the hybrid with T_m of 74 °C was obtained (data not shown). As shown in Fig. 1*a*, trace amounts of proinsulin mRNA (0.05–0.5 ng) could be measured by hybridisation with proinsulin cDNA. As it is difficult to prepare many islets from rat pancreas, this sensitive hybridisation assay is of particular value. To examine the specificity of the hybridisation reaction, we prepared total RNA from rat pancreatic islets, rat B-cell tumours and rat liver, and examined the hybridisation of proinsulin cDNA with total RNA from these tissues (Fig. 1*b*). From the extent of the hybridisation, the proinsulin mRNA content of total RNA from islets and that from B-cell tumours were determined to be 0.38% and 0.25%, respectively. As the proinsulin mRNA content of total RNA from islets and B-cell tumours determined by the hybridisation method was consistent with that determined by a wheat-germ cell-free translation assay[3–5], it is reasonable to assume that almost all the amount of proinsulin mRNA determined by the hybridisation method was translatable proinsulin mRNA. Total RNA of rat liver did not hybridise with proinsulin cDNA. These results suggest that the hybridisation reaction was highly specific.

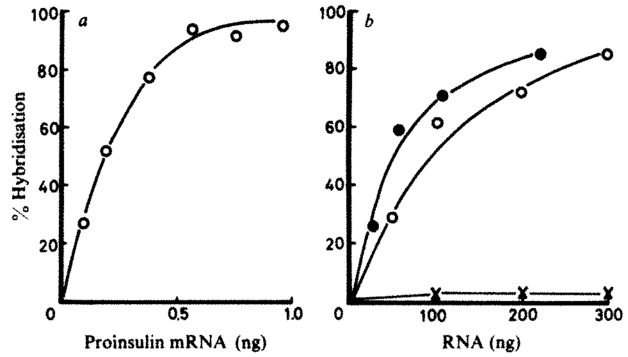

Fig. 1 Quantification of proinsulin mRNA by hybridisation with proinsulin cDNA. ^{3}H-proinsulin cDNA was synthesised using proinsulin mRNA purified from rat B-cell tumours as a template as described previously[3,4]. The hybridisation reaction was carried out at 65 °C for 20 h in a 50-μl reaction mixture (0.5 M NaCl, 25 mM HEPES-NaOH, *p*H 7.3, 1 mM EDTA, 0.1% SDS, 4 μg yeast RNA) after heating at 100 °C for 2 min. The reaction was stopped by adding 0.4 ml of 0.2 M sodium acetate (*p*H 4.5) containing 0.4 M NaCl, 2 mM zinc acetate and 6,000 units of nuclease S_1 (Seikagaku Kogyo), followed by incubation at 37 °C for 1.5 h. After incubation, 200 μg yeast DNA was added to the incubated mixture, and the nuclease S_1-resistant hybrid was precipitated with cold 10% trichloroacetic acid, and collected on a Toyo membrane filter TM-2. The filter was dissolved in 2 ml Cellosolve and the radioactivity was counted in a toluene-based scintillation solution. Total RNA was prepared from rat pancreatic islets, rat B-cell tumours and rat liver with the phenol extraction and LiCl-precipitation procedures[3]. *a*, Hybridisation of purified proinsulin mRNA with ^{3}H-proinsulin cDNA. *b*, Hybridisation of the total RNA from pancreatic islets (●), B-cell tumours (○) and liver (×) with ^{3}H-proinsulin cDNA.

The effects of glucose on proinsulin synthesis in isolated pancreatic islets of rats were examined. When the concentration of glucose in the incubation medium was increased from 2.8 mM to 25 mM, the proinsulin synthesis was greatly stimulated (~10-fold) (Fig. 2*a*) and proinsulin synthesis in the glucose-stimulated islets comprised 53% of the total protein synthesis. In contrast, the non-proinsulin protein synthesis was only slightly stimulated (~1.3-fold). These results suggest that the glucose-stimulation effect on protein synthesis in islets is highly specific to proinsulin synthesis.

In a wheat-germ cell-free system, we found that the amount of proinsulin mRNA in islets as determined by translation assay

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* To whom all correspondence should be addressed.

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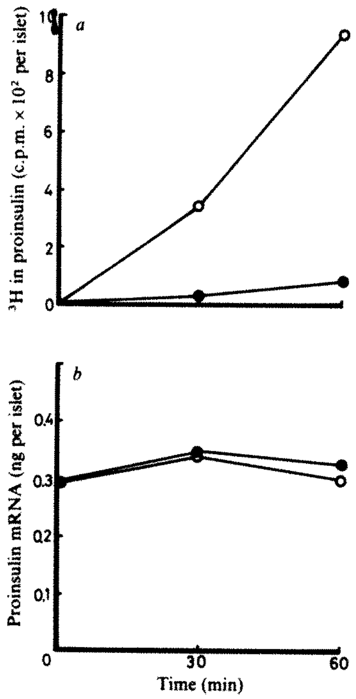

Fig. 2 *a*, Time course of induction of proinsulin synthesis by glucose in pancreatic islets. Pancreatic islets were prepared from male Wistar rats (200–240 g), which had been fasted for 18 h, in mild conditions of collagenase treatment as described previously[6]. Twenty islets were incubated at 37 °C for the indicated time in 125 μl Dulbecco's modified Eagle's medium (without glucose and leucine) containing 10 μCi ^{3}H-leucine (57.4 Ci mmol^{-1}, NEN), 2.8 mM (●) or 25 mM (○) glucose and 20% fetal calf serum (GIBCO). After incubation, the incubated islets were washed three times with 1 ml ice-cold Hank's solution, suspended in 0.2 ml of 60 mM Tris-phosphoric acid (*p*H 6.8) containing 1% SDS, 5% 2-mercaptoethanol and 10% glycerol, and sonicated for 10 s with a microtip of a Kontes ultrasonic cell disruptor. The sonicated material was heated at 100 °C for 2 min. An 80-μl aliquot of the heated sample was submitted to SDS-polyacrylamide (15%) gel electrophoresis (1 × 4 cm)[7]. After electrophoresis, the gel was cut into 1-mm slices and extracted with a Gilson B-100 gel fractionator. The radioactivity of the gel slice was counted in a toluene-based scintillation solution. The amount of newly synthesised proinsulin was calculated by summing ^{3}H-leucine radioactivity of the gel slices corresponding to the proinsulin peak[4,5]. *b*, Time course of proinsulin mRNA level during induction of proinsulin synthesis by glucose in pancreatic islets. Twenty-five islets were incubated at 37 °C for the indicated time in the presence of 2.8 mM (●) or 25 mM (○) glucose as described in Fig. 2*a*, except for addition of 0.2 nmol unlabelled leucine instead of 10 μCi ^{3}H-leucine. The incubated islets were washed once with 1 ml ice-cold Hank's solution and sonicated for 20 s with a microtip of a Kontes ultrasonic cell disruptor in 0.5 ml of 0.1 M Tris-HCl (*p*H 9) containing 10 mM EDTA, 0.1 M dithiothreitol, 2% SDS and 20 μg yeast RNA and an equal volume of a phenol solution (phenol/chloroform/isoamylalcohol 50 : 50 : 2) equilibrated with 0.1 M Tris-HCl (*p*H 9) containing 10 mM EDTA. The emulsion was vigorously shaken at room temperature for 5 min, left on ice for 5 min and centrifuged to separate two phases. The water phase was removed, and the phenol phase was re-extracted with 0.5 ml of 0.1 M Tris-HCl (*p*H 9) containing 10 mM EDTA and 2% SDS. The combined water phases were re-extracted with the phenol solution. Nucleic acid was precipitated by adding 0.1 vol. of 2 M sodium acetate (*p*H 5.5) plus 2.5 vol. of ethanol at −20 °C for 16 h. The precipitate was collected by centrifugation and washed once with 2 ml of cold 75% ethanol. Aliquots of the nucleic acid were used for quantification of proinsulin mRNA by hybridisation with proinsulin cDNA.

remained unchanged during the period of proinsulin induction by glucose for up to 60 min[4,5]. In the present paper, we have confirmed our previous result by the hybridisation method. The amount of proinsulin mRNA in the glucose-stimulated (25 mM glucose) or -unstimulated (2.8 mM glucose) islets remained unchanged during the period of proinsulin induction for up to 60 min (Fig. 2*b*).

To examine the subcellular localisation of proinsulin mRNA in the glucose-stimulated or -unstimulated islets, we prepared subcellular fractions from the islets. After pancreatic islets were incubated in a medium containing 2.8 mM or 25 mM glucose for 60 min at 37 °C, the islets were homogenised with rat liver as carrier, and membrane-bound polysome, free polysome and post-polysomal supernatant fractions were prepared. RNA was extracted from the subcellular fractions. Aliquots of the extracted RNA were used for quantification of proinsulin mRNA (Table 1). Recoveries of proinsulin mRNA in the membrane-bound polysome fractions were 58% for glucose-stimulated and 40% for unstimulated islets. However, a significant amount of proinsulin mRNA was also detected in the post-polysomal supernatant fraction; this was more marked in the unstimulated islets.

In some eukaryotic cells, some mRNAs have been shown to be present in a post-polysomal supernatant fraction as messenger ribonucleoprotein (mRNP; actin mRNP[9], histone mRNP[10], globin mRNP[11], albumin mRNP[12]). As it has been suggested that the mRNP may be involved in the regulation of the mRNA translation[9,12], it would be interesting to determine whether proinsulin mRNA is present in the post-polysomal supernatant fraction complexed with protein to form mRNP. To characterise the form of proinsulin mRNA present in the supernatant fraction, the supernatant was fractionated by sucrose gradient centrifugation and proinsulin mRNA sequences in each gradient fraction were quantified with proinsulin cDNA. The sedimentation profile of proinsulin mRNA sequences in the supernatant fraction exhibited an almost single major peak in the region of approximately 9S, which corresponds to the sedimentation value of intact proinsulin mRNA free from protein (Fig. 3)[3]. This suggests that proinsulin mRNA detected in the post-polysomal supernatant exists as an intact and free form.

The present experiment with isolated pancreatic islets of rats shows that the amount of proinsulin mRNA remains unchanged during the induction of proinsulin synthesis by glucose. Thus glucose regulates proinsulin synthesis at the translation level (translational control). Proinsulin mRNA, and mRNAs coding for other secretory proteins, have been shown to be translated on the membrane-bound polysome[13]. From the subcellular distribution of proinsulin mRNA in the glucose-stimulated or

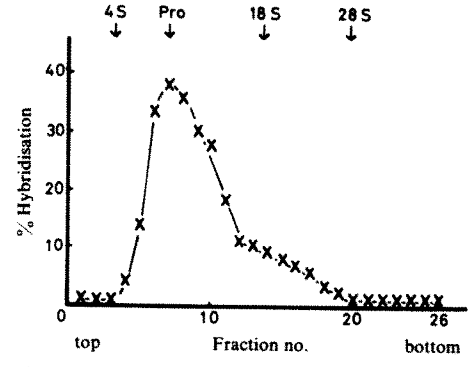

Fig. 3 Sucrose gradient sedimentation profile of proinsulin mRNA sequences in post-polysomal supernatant. After concentration with a Toyo membrane filter UK-50, an aliquot of the post-polysomal supernatant fraction of glucose-unstimulated islets was applied on a linear 15–30% sucrose gradient made in 10 mM Tris-HCl (*p*H 7.6) containing 0.5 M KCl, 2 mM EDTA and 100 μg ml^{-1} heparin. Centrifugation was performed at 60,000 r.p.m. at 0 °C for 3.5 h in a Beckman SW 60 Ti rotor. The gradient was fractionated with a Buchler Instrument Densi-Flow. From each fraction, RNA was extracted and used for quantification of proinsulin mRNA by hybridisation with proinsulin cDNA. Arrows indicated relative sedimentation of: 4S, rat liver tRNA; Pro, proinsulin mRNA; 18S, rat liver 18S rRNA; 28S, rat liver 28S rRNA.

Table 1 Subcellular distribution of proinsulin mRNA in pancreatic islets

Glucose	Membrane-bound polysome	Free polysome	Post-polysomal supernatant
Expt 1			
2.8 mM	0.099 (39.4)	0.033 (13.1)	0.119 (47.5)
25 mM	0.136 (57.9)	0.028 (11.9)	0.071 (30.2)
Expt 2			
2.8 mM	0.109 (41.4)	0.031 (11.8)	0.123 (46.8)
25 mM	0.145 (58.4)	0.036 (14.5)	0.067 (27.1)

One hundred and fifty islets were incubated at 37 °C for 60 min in the presence of 2.8 mM or 25 mM glucose as described in Fig. 2*a*, except for addition of 0.2 nmol unlabelled leucine instead of 10 μCi ^{3}H-leucine. The incubated islets were washed once with 1 ml ice-cold Hank's solution, added to 0.1 g wet weight of rat liver as carrier and homogenised at 0 °C with 10 strokes of a motor-driven glass–Teflon Potter–Elvehjem homogeniser in 3.9 ml of 250 mM sucrose containing 10 mM HEPES-KOH (*p*H 7.4), 75 mM KCl, 5 mM $MgCl_2$, 3 mM glutathione and 0.5% diethyl pyrocarbonate. The homogenate was centrifuged at 2,000 r.p.m. at 0 °C for 10 min in a Sorvall HB-4 rotor. After addition of heparin (100 μg ml^{-1}), membrane-bound polysome, free polysome and post-polysomal supernatant were prepared from the post-nuclear supernatant essentially according to the same methods as of Ramsey and Steele[8] and Bag and Sarkar[9]. Each subcellular fraction was precipitated with cold 10% trichloroacetic acid and washed with cold 75% ethanol and diethyl ether. Nucleic acid was extracted from the trichloroacetic acid-precipitate as described in Fig. 2*b*. Aliquots of the nucleic acids were used for quantification of proinsulin mRNA by hybridisation with proinsulin cDNA. Values are expressed as ng proinsulin mRNA per islet. The numbers in parentheses give the percentage of the post-nuclear supernatant.

-unstimulated islets, we can speculate as to possible mechanisms of translational control. Although the rate of proinsulin synthesis in the glucose-stimulated islets was 10 times that in the unstimulated islets, only a small difference between the amount of proinsulin mRNA in both membrane-bound polysome fractions was observed (58% and 40% in the stimulated and unstimulated islets, respectively). These results indicate that proinsulin synthesis is mainly regulated by enhancing the translation efficiency of proinsulin mRNA on the membrane-bound polysome.

A significant amount of proinsulin mRNA was also detected in the post-polysomal supernatant fraction (Table 1). The amount of proinsulin mRNA in the supernatant fraction was decreased by glucose stimulation. This decrease in proinsulin mRNA in the supernatant fraction was closely correlated with an increase in proinsulin mRNA in the membrane-bound polysome fraction. Therefore, the regulation by glucose of proinsulin synthesis in pancreatic islets is assumed to be achieved also in part by the transfer of proinsulin mRNA in the supernatant to the membrane-bound polysome.

Recent observations indicate that the rates of synthesis in proteins such as ovalbumin[14], globin[15], immunoglobulin[16], growth hormone[17], tyrosine aminotransferase[18] and tryptophan oxygenase[19] are closely correlated with the amount of the corresponding mRNA in cells. Therefore, the expression of genes of these proteins as well as many other proteins in eukaryotic cells is believed to be regulated at the transcription level.

The translational control of proinsulin synthesis by glucose as reported here seems to be a unique regulation of protein synthesis in eukaryotic cells. As insulin regulates the glucose level in the blood, the rates of both secretion and synthesis of insulin are required to change rapidly according to the change of the blood glucose level. Translational control may be a means of ensuring rapid regulation of proinsulin synthesis by glucose.

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A ρ-recognition site on phage λ *cro*-gene mRNA

Susan L. Bektesh* & John P. Richardson

Program in Molecular, Cellular and Developmental Biology, Department of Chemistry, Indiana University, Bloomington, Indiana 47405

The synthesis of RNA catalysed by RNA polymerase from *Escherichia coli* is terminated at specific sites on DNA templates through the action of a multimeric basic protein known as ρ (refs 1, 2). Three lines of evidence suggest that an interactions of ρ with the nascent RNA is important for this termination. First, ρ binds strongly to RNA[3]; second, ρ expresses an RNA-dependent ATPase activity which is essential for termination[4,5]; third, RNA polymerase does not terminate RNA synthesis at ρ-dependent sites when the nascent RNA is digested by ribonuclease during transcription[2,6]. From the fact that certain RNAs, particularly single-stranded, pyrimidine-rich polymers containing at least 10% cytidylate residues, are more effective than other RNAs at promoting ρ-ATPase, it has been proposed[7] that ρ recognises specific sites on a nascent RNA for its action. We present evidence here that ρ does bind at a specific region on a mRNA transcribed from bacteriophage λ DNA.

A precisely located site where the synthesis of an RNA molecule is terminated by ρ action is at a region, called t_{R1}, that is 92–96 base pairs to the right of the ochre translation termination triplet for the *cro*-gene sequence on λ DNA[8]. Transcripts initiated at P_R on λ and terminated in this region are 308–312-nucleotide-long RNAs and sediment at 8–9S (ref. 9). We call these, collectively, λ *cro* mRNA, and its sequence, as deduced from published information on the sequences of the λ DNA in the area of the *cro* gene and the 5′ and 3′ ends of the RNA, is given in Fig. 1.

To study the binding of ρ to a specific site on λ *cro* mRNA, the RNA is prepared by transcription *in vitro* of a λ DNA fragment containing the sequences from the restriction nuclease *Hin*dIII site at position 77.6 (in gene C_I) to the *Eco*RI site at position 81. This fragment actually codes for the synthesis of two RNAs *in vitro*, λ *cro* mRNA and λ *oop* RNA. From studies using a nitrocellulose filter binding assay, we have found that ρ forms a complex with λ *cro* mRNA, but not with λ *oop* RNA. We estimate that the association constant for the ρ–λ *cro* mRNA interaction is of the order of 10^8 M^{-1}. This binding is not high enough to isolate a fragment of RNA protected by ρ against

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* Present address: Department of Genetics, University of Washington, Seattle.

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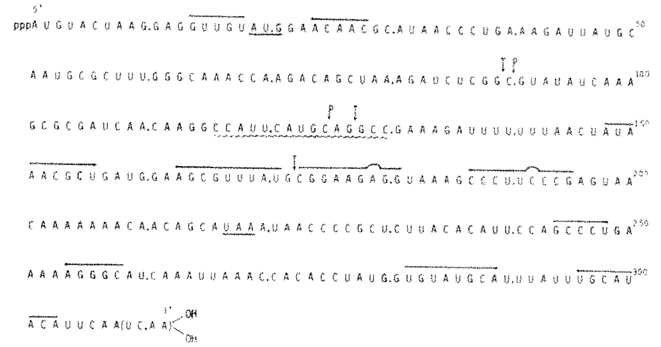
5'
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AACGCUGAUG.GAAGCGUUUA.UGCGGAAGAG.GUAAAGCCCU.UCCCGAGUAA^{200}
CAAAAAAACA.ACAGCAUAAA.UAACCCCGCU.CUUACACAUU.CCAGCCCUGA^{250}
AAAAGGGCAU.CAAAUUAAAC.CACACCUAUG.GUGUAUGCAU.UUAUUUGCAU^{300}
ACAUUCAA(UC.AA) 3' OH OH

Fig. 1 The nucleotide sequence of λ *cro* mRNA. This sequence is based on published information[8,11–13]. Dots are placed after every 10 residues. The parenthesis at the 3′ end indicates that termination occurs at any one of those bases. The AUG initiation codon and UAA termination codon for *cro* protein synthesis at residues 19–21 and 217–219, respectively, are underlined. The overlining with arrows designates some inverted sequence repetitions that might be involved in *cro* mRNA secondary structure. The symbols ⫯ and ꝭ indicate preferred sites of cleavage with T$_1$ and pancreatic ribonucleases, respectively. The wavy underlining indicates the region where bond cleavage is inhibited by ρ.

ribonuclease digestion; however, it is high enough for ρ partially to protect sites on the RNA against cleavage by ribonucleases. We use this effect to demonstrate that ρ binds to a specific site on λ *cro* mRNA.

To simplify the task of locating cleavage sites on λ *cro* mRNA, we use RNA labelled at the 5′ end and analyse the size distribution of its partial cleavage products by polyacrylamide gel electrophoresis. With this technique, the size of a labelled RNA fragment can be directly related to the point of cleavage on the primary structure of the *cro* RNA. λ *cro* mRNA labelled at the 5′ end was prepared by transcription of the λ *cro*-gene DNA fragment *in vitro* (with ρ present) using [γ-^{32}P]ATP. As λ *oop* RNA is initiated with GTP, the only labelled product is λ *cro* mRNA, which because of the heterogeneity in its termination, produces an unresolved group of bands on the gel rather than a single sharp band (Fig. 2, lanes 1 and 8). A partial digest of this RNA with pancreatic ribonuclease produces several distinct new bands, the two most prominent of which are at positions corresponding to RNA chain lengths of 90 and 125 nucleotides (lane 2). When the cleavage is carried out in the presence of a 10-fold molar excess of ρ over the RNA, many of the bands remain unchanged (lane 3), but the intensities of the bands from position 115 to position 129 are lower. Similarly, partial digestion of free λ *cro* mRNA with T$_1$ ribonuclease produces three very distinct bands (lane 6), including one at position 127 that is totally absent when the digestion is carried out in the presence of ρ (lane 7). The inclusion of ATP in the reaction mixture has no effect on the pattern of bands formed nor on the sites protected by the presence of ρ. (Lanes 4 and 5 show the results with pancreatic ribonuclease digestion with and without ρ present, respectively; the results with T$_1$ ribonuclease are not shown.)

The results show that there is a wide range of susceptibility of the bonds in λ *cro* mRNA to attack with ribonucleases. The most abundant products found after a limited partial digestion are the result of breakage at the most susceptible bonds. The very distinct pattern of products found is a clear indication that λ *cro* mRNA has a highly ordered secondary and perhaps tertiary structure. Presumably the most susceptible bonds are at exposed portions of the RNA. The presence of ρ clearly inhibits ribonuclease action at some susceptible points but not others. As T$_1$ ribonuclease, pancreatic ribonuclease and ρ are all more active on single-stranded RNAs than on double-helical RNAs, we expect ρ to bind to an exposed portion of the RNA that would also be a preferred site for ribonuclease action. Thus, the very specific inhibition caused by ρ is probably the result of a direct blockage of a cleavage site by the bound ρ molecule, rather than the result of a conformational change at the cleavage site induced by the binding of ρ to some other sequence of the RNA.

Because there are so few sites that are highly susceptible to cleavage, this experiment gives only limited information on the extent of the λ *cro* mRNA that could be in contact with ρ. The results suggest that there is some degree of contact with the region of residues 115–128, but that contact does not extend to position 88 on one side nor position 172 on the other side. With poly(C), ρ can bind up to 60 nucleotides in a single stretch[10]. However, as the binding of ρ to λ *cro* mRNA is several orders of magnitude weaker than its binding to poly(C), the extent of contact could involve many fewer nucleotides.

Based on an examination of the primary structure of λ *cro* mRNA, we predicted that a likely site for ρ binding would be the pyrimidine-rich segment of RNA on the part of the untranslated

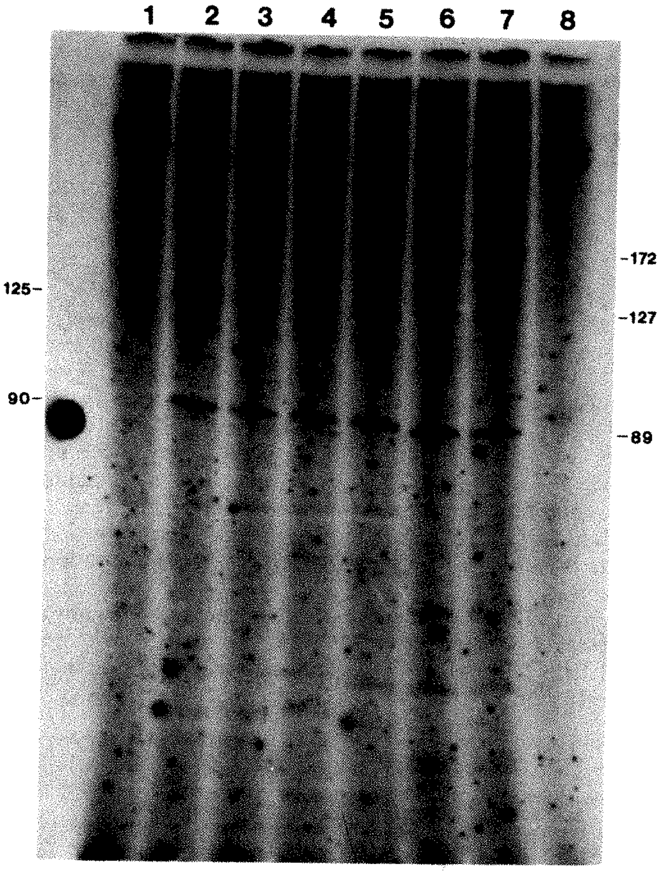

Fig. 2 Effect of ρ on the cleavage of λ *cro* mRNA with pancreatic and T$_1$ ribonucleases. [5′-^{32}P]-labelled λ *cro* mRNA was synthesised *in vitro* in a reaction mixture containing 5.6 pmol *E. coli* MRE 600 RNA polymerase holoenzyme, 5.6 pmol ρ protein, 1 pmol λ *cro* DNA fragment (77.6–81.0 map units of λ DNA), 0.1 mM each [γ-^{32}P]ATP (10–20 Ci mmol^{-1}), GTP and UTP, 0.05 mM CTP, 40 mM Tris-HCl, *p*H 8, 10 mM MgCl$_2$, 50 mM KCl, 0.1 mM dithiothreitol and 0.1 mM EDTA in a total volume of 0.1 ml. After incubation for 20 min at 37 °C, the RNA was purified by extraction successively wipenol and with a chloroform/isoamyl alcohol mixture (24 : 1) and precipitated with 2 volumes of 95% ethanol. After centrifugation for 30 min at 20,000*g*, the pelleted precipitate was washed in 0.5 ml 95% ethanol and the washed precipitate dissolved in a solution containing 10 mM Tris-HCl, *p*H 7.4, 10 mM KCl, 10 mM MgCl$_2$, 0.1 mM dithiothreitol, 0.1 mM EDTA, bovine serum albumin (50 μg ml^{-1}) and *E. coli* tRNA (1 mg ml^{-1}). The samples analysed contained, in 10 μl total volume, 0.1 pmol of [5′-^{32}P]λ *cro* mRNA (~10,000 c.p.m.). Samples with ρ contained 0.4 μg and were preincubated for 1 min at 37 °C; ATP, when present, was 1 mM. Ribonuclease treatments at 37 °C were initiated by adding 1 μl of either T$_1$ ribonuclease (Sankyo-Calbiochem, 80 units ml^{-1}) or pancreatic ribonuclease (Worthington, 5 × 10^{-3} units ml^{-1}) and were terminated after 15 min by the addition of 1 μl 1% diethyl oxydiformate. After adding 10 μl of a solution containing 40% sucrose, 0.1% xylene cyanol and 0.1% bromphenol blue, the samples were applied to a 10% acrylamide gel (14 cm × 25 cm × 0.15 cm) containing 7 M urea in 90 mM Tris, 90 mM boric acid, 4 mM EDTA and 0.1% SDS. After electrophoresis for 5 h at 350 V, the lowest section of the gel containing residual unincorporated [γ-^{32}P]ATP was cut away and the rest of the gel was washed in H$_2$O for 0.5 h (to remove urea), dried and exposed to Kodak NS-2T X-ray film in the presence of a Cronex Lightning-plus intensifying screen at −70 °C. Lanes 1 and 8, untreated RNA; lanes 2–5, RNA treated with pancreatic ribonuclease. ρ was present in samples shown in lanes 3 and 4 and ATP was present in samples shown in lanes 4 and 5. Lanes 6 and 7, RNA treated with T$_1$ ribonuclease in the absence and presence of ρ, respectively. The dark spot at the left marks the position of the xylene cyanol dye.

tail of the RNA that starts after the ochre chain termination triplet at position 220 and runs to the start of the proposed hairpin loop structure (*nut*R [see ref. 8]) at position 244. By itself, this region would probably have very little secondary structure. Although there is evidence that bonds from residues 220 and 244 are cut with pancreatic ribonuclease, the sites are not as sensitive as those at positions 90 and 125, and the single G residue at position 228 is resistant to T_1 ribonuclease action. Thus, it is likely that this stretch of RNA is protected by folding of the RNA. As ρ has no obvious effect on the activity of pancreatic ribonuclease in that region, it may be that the folding of the RNA prevents it from being an effective ρ binding site as well.

The results presented here indicate that ρ does bind to a specific site on λ *cro* mRNA and that is within the sequence coding for the *cro* protein 180 residues from the 3′ end rather than on the untranslated tail. In the current models[2,7,10], it is proposed that ρ protein functions in termination primarily to effect the release of RNA molecules from a transcription complex in which the RNA polymerase has paused at a termination site on the DNA template. As the sites for pausing are apparently determined by the interaction between the RNA polymerase, DNA and the nascent RNA[8], the initial site of attachment of the release factor need not be at a sequence adjacent to the 3′ end. Thus, the binding site detected in this study could be relevant to ρ function in termination. However, because the structure of RNA is very sensitive to the environment, which is not the same for a nascent RNA as it is for an isolated RNA, we cannot be certain that ρ binds to the same region on the nascent RNA. Hence, although we have identified for the first time a specific site for the interaction between ρ and a mRNA, further experiments will be needed to show whether this site is recognised by ρ during the process of transcription termination.

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Potential of *Escherichia coli* isolated from nature to propagate cloning vectors

James P. Robeson*, Raúl M. Goldschmidt & Roy Curtiss III

Department of Microbiology, Medical Center, University of Alabama in Birmingham, Birmingham, Alabama 35294

Concern has been shown in the scientific community with regard to the potential risk that the transmission of foreign genes from *Escherichia coli* K12-cloning vector systems to other microorganisms might represent[1]. Because of this we have attempted to evaluate the frequency in nature of *E. coli* strains that can serve as recipients and/or hosts for genetic information harboured by various *E. coli* cloning vectors used in recombinant DNA research. We assumed that genetic transfer from *E. coli* K12 to other *E. coli* strains was more likely than to bacteria of other species or genera, and consequently examined natural *E. coli* isolates for traits that would reflect their ability to propagate and/or maintain cloning vectors. In particular, we were interested in assessing the role that transduction might have in the dissemination of cloned genetic information. We also wanted to determine whether naturally occurring strains could acquire and stably maintain phage λ derived cloning vectors that generally contain *amber* mutations in essential genes[2–4]. By examining natural bacterial populations we attempted to evaluate the fitness of auxotrophic mutations that are generally introduced into *E. coli* K12 hosts used in recombinant DNA experiments. Therefore, we determined among natural isolates of *E. coli* frequency of strains exhibiting: (1) sensitivity to phages P1 and λ; (2) *amber* suppressor mutations; and (3) auxotrophic mutations. The results presented here allow an estimation of the feasibility and limitations of the successful spread among *E. coli* strains in nature of recombinant DNA molecules contained in particular *E. coli* K12 hosts.

In terms of propagation of cloning vectors, we considered it important to evaluate the possiblity that they might be transferred to and among natural *E. coli* strains through generalised transduction by a phage like P1. Although P1 is known to have a wide host range[5,6], the prevalence of P1-mediated transduction in nature, and the incidence of P1 sensitivity among natural isolates of *E. coli* are not known. We studied this last parameter for an indication of the importance which P1 transduction might have on the dissemination of plasmid cloning vectors among naturally occurring bacteria. Sensitivity to P1 of the *E. coli* isolates studied was determined using the phage derivative P1CM*clr100* (ref. 7) which confers resistance to chloramphenicol (Cm) to lysogenised cells. A substantial majority of the *E. coli* strains studied (about 70%) were found to be sensitive to P1 (Table 1, *c*). Generally these strains were lysogenised by P1CM*clr100* at frequencies ranging from 10^{-2} to 10^{-4}.

In the case of λ cloning vectors an inherent safety feature seems to derive from the low incidence of sensitivity to λ found among natural isolates of *E. coli* using a plaque formation assay[4]. However, these studies may have underestimated the sensitivity to λ of those strains, as plaque formation requires successful propagation of the phage and is therefore affected by factors such as reduced adsorption, restriction and/or the presence of a homoimmune phage[8], all of which will reduce the plating efficiency of λ. Considering these limitations to using lysis as a test for λ-sensitivity, we decided to examine our *E. coli* isolates for sensitivity to phage λ using the thermoinducible derivative λ*cam105* (ref. 9) which contains in its genome the determinant for Cm resistance, Tn*9*.

By these means, penetration of λ into λ-sensitive *E. coli* cells can be detected by the acquisition of a Cm-resistant phenotype that might result from lysogenisation by λ*cam105* and/or by transposition of Tn*9* to the chromosome, or a resident plasmid, or phage of the strain being studied. About 2% of the *E. coli* strains tested yielded Cm-resistant clones when infected with λ*cam105* (Table 1, *d*). These clones were analysed further and it was found that in eight out of nine instances, the Cm-resistant derivatives could grow at 30°C but not at 42 °C. This indicated that they were lysogens of λ*cam105* since the parental strains showed unimpaired growth at 42 °C. In addition, lysates prepared from the Cm-resistant derivatives which were thermosensitive could only transduce to Cm resistance *E. coli* K12 strains that were not λ-lysogens. The frequency of lysogenisation by λ*cam105* was estimated to be between 10^{-3} and 10^{-4}. The strain that gave rise to Cm-resistant clones which were not thermoinducible might represent a case in which λ was lost after infection, and the Cm-resistant phenotype resulted from a Tn9 transposition event. We noted that the λ derivative λ*vir* (ref. 10) did not form plaques on any of the strains shown to be infectable by λ*cam105*. This indicates that a plaque assay is generally not

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* Permanent address: Universidad Católica de Valparaíso, Chile.

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Table 1 Characteristics of natural isolates of *Escherichia coli*

Origin[a]	No. of isolates	Fraction of isolates sensitive to phages		Fraction of isolates with *amber* suppressor mutations[f]	Fraction of isolates containing auxotrophic mutations[i]
		P1[c] and	λ[d]		
Infections	325	212/310	2/237	2/254[g]	37/325
Stools	190	135/180	7/143	0/154	11/188
Polluted water	35	20/23	0/34	0/35	3/35
Totals	550	367/524	9/414[e]	2/443[h]	51/548
(%)		(70.0)	(2.1)	(0.4)	(9.3)

a, *E. coli* strains from infections were obtained from CDC stocks (Atlanta, Georgia) and additional strains were isolated from specimens collected at the UAB hospitals. All strains from infections were of human origin. Strains from faecal samples from both healthy and diseased human individuals were also obtained from UAB hospitals and CDC, respectively. *E. coli* strains were isolated from polluted water samples collected from wells and water systems around the Birmingham area. The strains from CDC (328 isolates) had been originally collected between 1972 and 1976. In most cases, the O, H and K antigenic types had been determined. Strains from UAB (187 isolates) and from polluted waters were collected during 1977. No serotyping was done with these strains.

b, The total number of isolates includes strains that were resistant to one or more of the following drugs: Ap, Tc, Km and Cm (Sigma). Some of these strains, depending on the resistance(s) they displayed, were not tested for *amber* suppressors and/or sensitivity to phages P1 and λ due to experimental design.

c, Lysates of P1CM*clr100* were prepared by thermoinduction of the *E. coli* K12 P1CM*clr100* lysogen χ1932 (F$^-$ *tsx* λ$^-$ *nalA rpsL*). *E. coli* strains to be tested were grown at 37 °C to a density of 1×10^8 cells ml^{-1} in L broth[13] supplemented with 2.5×10^{-3} M CaCl$_2$. Cultures were infected with P1CM*clr100* at room temperature using the phage at a multiplicity of infection of 1. After allowing 20 min of preadsorption at 30 °C, samples of the infected cultures were plated on Penassay agar (PA, Difco) supplemented with 12.5 μg ml^{-1} Cm. After 24–36 h of incubation at 30 °C, plates were examined for the presence of Cm-resistant lysogens.

d, Lysates of λcI857*cam105* were prepared by thermoinduction of its *E. coli* K12 host MGBO (F$^-$ *thi rha trkA*$_{trkD}$) (provided by L. A. MacHattie). λ*cam105* was then used to detect λ-sensitive strains using the same procedure described for P1-sensitivity screening, except that the cells were grown in L broth without glucose and supplemented with 1×10^{-2} M MgSO$_4$. Also, selective plates contained 20μg ml^{-1} Cm.

e, Strains lost on storage at 4 °C account for the lower number of isolates tested for λ-sensitivity in relation to the number examined for P1-sensitivity.

f, See text for experimental rationale. *E. coli* χ1776 (F$^-$ *tonA53 dapD8 minA1 supE42* Δ40[*gal-uvrB*] λ$^-$*minB2 rfb* – *2 nalA25 oms* – *2 thyA142 metC65 oms* – *1* Δ29[*bioH-asd*] *cycB2 cycA1 hsdR2*)[14] containing the plasmid pLM2 [Ap(am) Tc(am) Km][11] was used as donor in matings with the natural *E. coli* isolates tested. χ1776 (pLM2) and the recipient strain under examination were grown to a density of 1×10^8 cells per ml in L broth supplemented with 100 μg diaminopimelic acid (DAP) and 40 μg thymidine (Thd) per ml. Samples (0.1 ml) of donor and recipient were mixed on PA plates containing DAP and Thd and incubated overnight at 37 °C. Generally, the recipient strain was heat shocked before mating to minimize restriction of the incoming plasmid[15]. After incubation, the mating mixture was resuspended in BSG[16] and samples were plated onto PA containing 100 μg ml^{-1} Km to select pLM2 transconjugants. Selection against the donor was achieved by omitting DAP and Thd in the PA Km plates. A minimum of 5 pLM2 transconjugants of each natural *E. coli* strain tested were examined for their ability to grow in the presence of Ap (25 μg ml^{-1}) and/or Tc (25 μg ml^{-1}) to determine if the *E. coli* strain being studied contained an *amber* suppressor function.

g, The two strains showing *amber* suppressors were of human origin and had been originally recovered from infectious episodes of the urinary tract and blood. The strains were stocked at CDC with the numbers 3764–73 (urine isolate) and 3885–74 (blood isolate). Their serotypes were O undetermined: H4 and O16: H6, respectively. Both were prototrophs, P1-sensitive and λ-resistant. Both strains constituted homogeneous *amber* suppressor populations.

h, No. of strains into which pLM2 was successfully transferred to test for *amber* suppressors. The actual number of isolates that could have been tested for suppressors was 486 out of the 550 originally collected.

i, The auxotrophic character of the strains examined was determined by their inability to grow on minimal agar[16] plates with 1% glucose as carbon source.

sensitive enough to determine whether a particular *E. coli* strain can be infected by λ or not.

It was mentioned above that phage λ-derived cloning vectors generally possess *amber* mutations in essential genes[2–4]. It has also been proposed (but not yet accomplished) to construct non-conjugative plasmid cloning vectors with the same type of mutations. The purpose of constructing vectors with *amber* mutations is to confine their full expression to an *E. coli* K12 host with an *amber* suppressor. This approach to the development of safer vectors for cloning was obviously based on the assumption that the majority of bacterial strains found in natural environments would not contain *amber* suppressors and thus would not propagate cloning vectors should they accidentally acquire them. However, no data are available on the incidence of natural bacterial strains possessing *amber* suppressor mutations. We sought to detect such mutations among the *E. coli* isolates collected using the plasmid pLM2 (ref. 11). This plasmid is a derivative of RP1, a broad host range plasmid that specifies resistance to ampicillin (Ap), tetracycline (Tc) and kanamycin (Km). pLM2 differs from RP1 in that the Ap and Tc markers have *amber* mutations. Consequently, these antibiotic resistances are expressed only in a host strain that contains an *amber* suppressor. Therefore, pLM2 was used to detect *amber* suppressors by transferring it to the test strains and verifying whether resistance to Ap and Tc was expressed. We could transfer pLM2 to 91% of the strains that were suitable to be tested for *amber* suppressors (Table 1, *f*, *h*). Only two *E. coli* strains (representing about 0.4% of the total examined) were found containing *amber* suppressors. Both suppressor strains were detected among a group of isolates obtained from the Center for Disease Control (CDC), Atlanta, Georgia (see Table 1 *g*, for additional information).

Generally, *E. coli* K12 derivatives used as hosts in recombinant DNA experiments have specific auxotrophic mutations that result in a decrease in viability of the strains when they are placed in laboratory conditions that mimic a natural environment. Examples of mutations that severely hinder survival of *E. coli* K12 derivatives are those that lead to the requirement of cell wall components like diaminopimelic acid and/or precursors of nucleic acids like thymidine. An independent way of assessing the effect of such mutations on the survival of a particular natural strain is to determine whether the corresponding phenotypes are present among natural bacterial isolates. Therefore, we tested our *E. coli* isolates for the presence of auxotrophic mutations and whenever possible the nature of the metabolic requirement was determined. We found that approximately 9% of the natural *E. coli* strains studied were auxotrophs (Table 1), a result that is in agreement with available information[12]. When further analysed for the nature of their nutritional requirement, 77% of the auxotrophic strains were found to require niacin. The rest required either a different vitamin (thiamine, pantothenate) or certain amino acids, namely cysteine or methionine. No purine or pyrimidine requirers were found. We noted that a group of *E. coli* isolates from urinary tract infections showed the highest incidence (25%) of auxotrophic strains.

The results of this investigation suggest that P1-mediated transduction might be an important mechanism for dissemination of genetic information in nature in view of the high incidence of P1-sensitive strains found among the *E. coli* isolates that were examined. This observation is consistent with the broad host range of P1 (refs 5, 6). However, additional experiments are necessary to evaluate further the possible role of P1 in the transmission of genetic material between natural bacterial populations. It would be desirable to determine titres of P1-like phage in nature, either as a free phage or as a prophage in lysogenic cells; in addition, stability and infectivity data of P1-like phage particles in natural conditions would be required. This information would be important in view of results that show that P1 is able to transduce plasmids similar to those used as cloning vectors, although at a lower frequency than for chromosomal genes (R. Goldschmidt and M. Inoue, unpublished).

In terms of λ cloning vectors, our observations indicate that these cloning vehicles have inherent safety features that derive from the relatively low incidence of λ-sensitive strains among naturally occurring strains of *E. coli*. From the results that indicate the presence at low frequency of *amber* suppressor mutations among the *E. coli* isolates examined, it is apparent that cloning vectors with *amber* mutations in essential genes contained in bacterial hosts with suppressors, offer a safety advantage over vectors lacking *amber* mutations. We estimate that the probability of successful expression of an *amber*-containing cloning vehicle would be reduced at least 100-fold when compared to that of a vector without such genetic lesions in its genome.

The fact that we were able to detect auxotrophs among the bacterial strains tested suggests that certain vitamin and amino acid requirements do not necessarily represent a selective disadvantage to the bacteria that possess them. On the other hand, the diversity of auxotrophic mutations was rather limited and it seems unlikely that all possible types of auxotrophs will be found among natural bacterial isolates. In particular and as expected, we did not find any of the phenotypes associated with mutations that result in a severe impairment of the ability of *E. coli* K12 derivatives to survive in natural environments.

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Anisotropy of Young's modulus of bone

J. Lawrence Katz*

Department of Orthopaedic Surgery, Harvard Medical School and The Children's Hospital Medical Center, Boston, Massachusetts 02115

Bonfield and Grynpas[1] have compared their experimental data for Young's modulus of elasticity versus the angle of orientation of the specimen to the long axis of bone with a theoretical curve predicted from a calculation for fibre reinforced materials proposed by Currey[2]. As a result of the poor agreement between the two curves they conclude "... an alternative model is required to account for the dependence of Young's modulus on orientation" (ref. 1). Such an alternative has been under development in my laboratory for the past 8 years (refs 3–7). It is a two-level hierarchical fibre-reinforced composite model which appears to provide a much more suitable description of the behaviour of bone as a fibre-reinforced composite material.

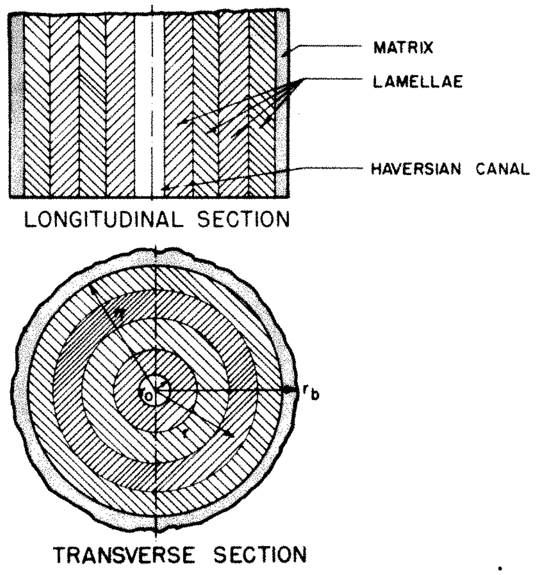

Fig. 1 A single osteon modelled as a hollow fibre for the Hashin–Rosen[9] calculation. r_0 is the radius of the haversian canal (hollow), r_f is the radius of the single osteon (fibre) and r_b is the radius of the osteon plus cement line (fibre and matrix).

At the first level of the model, a single osteon is treated as a coherent entity, modelled as a hollow, right circular cylinder comprised of concentric lamellae surrounding the haversian canal (Fig. 1). On the second level the osteons or interstitial lamellae are assumed to be packed in a pseudo-hexagonal arrangement (Fig. 2), as suggested for compact cortical bone by Yoon and Katz[8] based on microscopic observations and ultrasonic wave propagation measurements.

The Hashin–Rosen calculation[9] for hexagonally and randomly (nearly hexagonally) arrayed hollow fibre-reinforced composites can now be applied directly. In this model only five independent effective elastic constants are required to characterise completely the anisotropic elastic properties of the material, either exactly as in the case of hexagonal arrays or approximately for random (nearly hexagonal) arrays. Variational techniques provide a set of bounds for the five effective

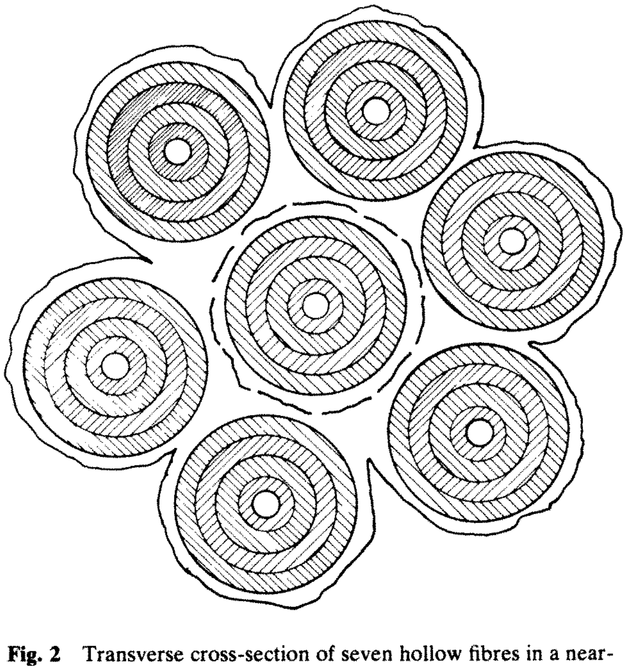

Fig. 2 Transverse cross-section of seven hollow fibres in a near-hexagonal array modelling a specimen of cortical haversian bone. (The number of lamellae depicted for each osteon is for artistic purposes only and does not affect the calculation.)

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* Permanent address: Center for Biomedical Engineering, Rensselaer Polytechnic Institute, Troy, New York 12181.

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elastic constants; the upper bounds are obtained by using displacement criteria, the lower bounds by using the stress-vector criteria.

Two kinds of input are required for this calculation. First, geometric factors are needed that include the ration of the transverse area of the haversian canal to that of the osteon, given by r_0^2/r_t^2 (Fig. 1) and denoted by V_0, and the ratio of the transverse area of the osteon to osteon plus matrix, given by r_t^2/r_b^2 (Fig. 1) and denoted by V_t. Second, mechanical properties are needed that include the Young's modulus and Poisson's ratio for both the fibre represented here by the single osteon (E_{OST} and ν_{OST} respectively) and the matrix represented here by the ground substance (E_{GS} and ν_{GS} respectively). Reported values of E_{OST} under various ultrastructural configurations[4] agree well with values for single osteons measured by Frasca[10]; values of ν_{OST} have also been derived from his data. Values for the ground substance have been inferred from the angular dependent properties of bone obtained from ultrasonic experiments[8].

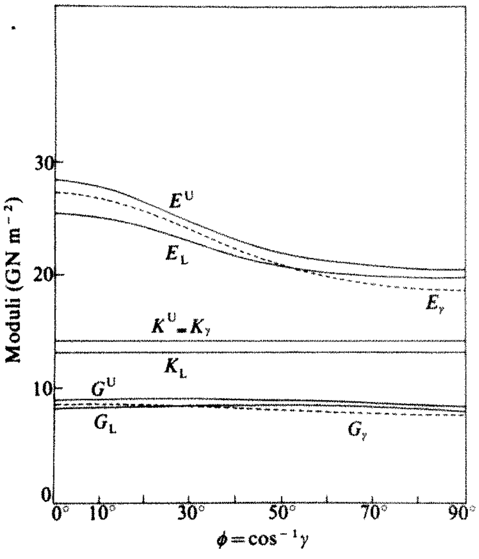

Fig. 3 The angular dependent variation of the elastic moduli for human cortical bone with respect to the orientation of the long axis of the bone (ϕ) calculated from the ultrasonic data of Yoon and Katz[8] (Young's modulus, E_γ; bulk modulus, K_γ; and shear modulus, G_γ, are denoted by - - -) compared with the theoretical curves representing the upper and lower bounds on the angular dependent behaviour of the elastic moduli using the Hashin–Rosen calculation[9]. (Young's moduli, E^U, E_L; bulk moduli, K^U, K_L; and shear moduli, G^U, G_L respectively are denoted by ——.) Input parameters are: $E_{OST} = 40.0$ GNm^{-2}; $E_{GS} = 10.0$ GN m^{-2}; $\nu_{OST} = 0.30$; $\nu_{GS} = 0.25$; $V_0 = 0.10$ and $V_T = 0.70$.

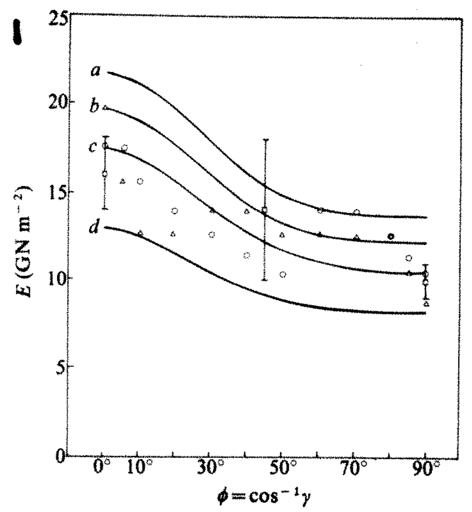

Fig. 4 The variation of Young's modulus for bovine femoral specimens with respect to the orientation of specimen axes cut at the angle ϕ to the long axis of the bone for wet (○) and dry (△) conditions from the work of Bonfield and Grynpas[1] compared with the angular dependent behaviour of Young's modulus (——) computed using the Hashin–Rosen calculation[9]. Each curve represents a different lamellar configuration of collagen fibres and hydroxyapatite crystallites within a single osteon. *a*, 65% longitudinal fibres; *b*, 57% longitudinal fibres; *c*, 50% longitudinal fibres; *d*, 37%% longitudinal fibres. Other input parameters are: $E_{GS} = 5.5$ GN m^{-2}; $V_0 = 0.20$; and $V_T = 0.80$.

These ultrasonic measurements have yielded five independent elastic stiffness constants which also can be used to calculate the technical moduli as a function of the angular orientation with respect to the stress axis. The angular dependence of the Young's E, bulk, K, and shear, G, moduli calculated from the ultrasonic data are compared on Fig. 3, with a set of upper and lower bound curves generated by using the best pair of five effective elastic constants obtained from a large number of sets computed using the Hashin–Rosen formalism[9]. The values of these ultrasonic moduli for dry bone are all greater than those obtained by mechanical testing or low frequency sound measurements on wet bone (Fig. 4). This is due partly to the fact that dry bone is stiffer and more rigid than the same sample of bone when wet, and partly to the strain rate dependent behaviour of bone which results in increased stiffness with increase in strain rate[11]. Several groups of such curves have been analysed in detail to obtain representative sets of values for both the Young's modulus and Poisson's ratio of the matrix based on scaling the ultrasonic data to the mechanical and low frequency sound measurements.

Various combinations of these parameters have been used in calculations, based on the Hashin–Rosen formalism, which then generate sets of the five effective elastic constants. These constants are then used to calculate the angular dependence of such technical moduli as the Young's and shear moduli as a function of angular orientation with respect to the stress axis. These calculations have been used as the basis for determining which sets of parameters were used in generating curves *a–d* (Fig. 4) in order to model the data of Bonfield and Grynpas. Additional confirmation of this type of angular dependent behaviour for the elastic properties of bovine bone is shown in Fig. 4. The values measured for bovine bone specimens cut at 0°, 45° and 90° to the bone axis (denoted by the open squares with error bars) are from ref. 12.

Although this model simulates the behaviour of bone quite closely it cannot provide an exact description. It handles only one size of osteon in a given calculation. However, it can be adjusted to yield an average behaviour or to predict the upper and lower bounds on the elastic behaviour by the choice of lamellar micro- and ultrastructure (number of lamella, size of the haversian canal, fibre orientations and so on) as is shown in part by Fig. 4.

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MATTERS ARISING

Rotating lunar globules

SEVERAL theories have been proposed for the origin of the shapes of lunar dumbbell and ellipsoidal glass globules. The rotational theory[1] assumes that they solidify while rotating in free fall above the lunar surface, the rotation giving the elongation in form. A second theory imagines solidification of a jet of liquid again above the lunar surface and at some time during the break-up of the jet[2-4]. More recently Chernyak and Nussinov[5] have proposed that the shapes are caused by the sudden freezing of a vibrating droplet. They also cite arguments against a rotational origin. However, there is now much evidence favouring the rotational hypothesis.

Chernyak and Nussinov propose that "the drops vibrate" and "because of the exponential dependence of viscosity on temperature the particle solidifies and memorises its shape". Such a proposal is completely untenable as a glass drop cannot pass from an essentially liquid to solid state in a time that is short compared with its vibrational period. The period of vibration of a droplet of diameter 100 μm is about 10^{-4} s. Even assuming it radiates as a perfect black body the drop in temperature of such a globule during its vibrational period is between 0.1 and 0.01 K. Although the viscosity of glasses varies very rapidly with temperature (three orders of magnitude for a temperature change of 100 K) it is impossible that there can be any appreciable change in viscosity during a single period of oscillation, let alone during the small fraction of the period required by the vibrational hypothesis. Similar numerical analyses for larger or small droplets yield essentially the same conclusion because the ratio of the vibrational period to the time taken to cool through a given temperature interval varies only slowly with particle size[1] (in fact, as the square root of its linear dimensions).

Arguments by Chernyak and Nussinov[5] against the rotation hypothesis depend on the instability of dumbbell shapes and the effect of Coriolis forces which would bring about S or crescent shaped particles. Although several curved smooth glassy particles have been found in lunar soil and it is reasonable to use a Coriolis force mechanism to explain their shape, if the initial temperature of a particle is sufficiently high, the cooling time is long enough for viscous forces to damp out all appreciable internal motion. While still liquid the globule will thus eventually rotate as a rigid body[1] and Coriolis forces are then not involved.

Chernyak and Nussinov's argument that dumbbell shapes are unstable is also not substantial. Imagine a small liquid sphere in an inertial frame held together only by surface tension forces. Suppose a small external couple acting for a limited time causes the globule to be brought into rotation. After the couple has been removed the globule will attain an aspherical rotating form in which any internal motions will gradually be eliminated by viscous damping. The form of the globule is then stable yet not spherical. The process may be repeated so that more and more angular momentum will be slowly given to the globule. Eventually the system will disintegrate but it is unclear whether before this disintegration occurs stable forms may exist with re-entrant shaped cross-sections—dumbbells.

There are many other reasons for preferring the rotational hypothesis. (1) The photographed shapes of lunar dumbbells[6] are not the same as those formed by jet break-up[7]. The latter have low curvature at their extremities. (2) The curvature of the surfaces of lunar dumbbells has been shown to agree well with that predicted from rotational theory[6]. (3) The break-up of a jet is a transitory phenomenon: a rotating dumbbell is a stable form so that the latter would be expected to occur more frequently. (4) There is no evidence that regular cylindrical liquid jets can be formed by volcanic activity or hypervelocity impacts. Such a jet is required by the jet break-up hypothesis[2,3]. Some evidence does exist for jet formation following meteorite impact[8] but here 'jet' simply implies a group of particles moving with high speed in approximately the same direction. The confusion is semantic. (5) The angular momentum of dumbbells and prolate globules is well accounted for by local inhomogeneities in surface rock at the impact site. (6) Voids both in the dumbbells and the oblate spheroids tend to be found close to the rotational axis, whereas darker and more dense particles are found closer to the extremities[9]. Generally more migration has occurred in the case of the larger particles and voids; and this is precisely what would be expected from Stokes' law and the rotational hypothesis. (7) The densities of the dumbbell extremities are found to be higher than the central portions, a result predicted by the rotational hypothesis (T. Gold, personal communication).

The rotation hypothesis supplies a means of determining the maximum temperature of droplets during their formation. It also favours a lava fountain origin for many of the green and orange lunar droplets as no dumbbell-shaped globules have been reported among these samples[10]. It is also relevant to the question raised by Butler[11] concerning whether these droplets were splashed from molten lava by impact.

J. A. BASTIN

Department of Physics,
Queen Mary College,
University of London,
London E1, UK

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Dihydroergocryptine binding and α-adrenoreceptors in smooth muscle

THE recent letter by Kunos *et al.*[1] in which they "dissociate phentolamine-suppressible ^{3}H-dihydroergocryptine (^{3}H-DHEC) binding and α-receptors" in rabbit myometrium disagrees with previous reports[2,3] and contains several puzzling features.

First, the authors assume that the concentrations of phenoxybenzamine (POB) inhibiting noradrenaline-stimulated contractility should coincide with concentrations of POB that compete for ^{3}H-DHEC binding sites. As the number of steps between receptor binding and contractility is unknown, one ought not necessarily expect such different experimental approaches to yield similar results[4,5]. Moreover, it is probably unreasonable to ascribe the effects of a partially irreversible α-adrenergic blocker (POB) on contractility solely to blockade of α-adrenergic receptors, because POB also inhibits response to serotonin, histamine and acetylcholine[6]. Second, the authors suggest that two classes of ^{3}H-DHEC binding site (K_d values 1.5 and 5.5 nM) exist in their preparations. The data used to determine those K_d values were apparently obtained from pooled determinations of different uterine preparations. In view of the likely heterogeneity between particulates of crude myometrial homogenates subjected to

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five to seven centrifugations (Fig. 1 legend[1]), selective pooling of such results is an unreasonable approach for determining two classes of site with such similar K_d values.

Third, we find that the concentration curve of ^{3}H-DHEC binding for 'control' values plotted by Scatchard analysis of these data yields a single class of ^{3}H-DHEC site ($K_d = 4.6$ nM, $B_{max} =$ 85.9 fmol per mg, $r = 0.93$). Even if the authors were correct in defining two classes of site, we calculate that in experiments carried out at 2 nM, their "high affinity sites" contribute 55% and the "low affinity sites" 45%, and in studies at 8 nM, the high and low affinity sites contribute 35% and 65%, respectively. We have constructed theoretical binding curves for two-non-cooperative sites with values for K_d and B_{max} described by the authors and find no break such as shown by Kunos *et al.* Although positive cooperativity at the low affinity class of sites might generate such a break, we have never seen such positive cooperativity in over 50 ^{3}H-DHEC binding studies with rabbit myometrium preparations.

Moreover, as binding at "high" (>5 nM ^{3}H-DHEC) concentrations is the sum of high and low affinity binding sites, the reported inhibition of ^{3}H-DHEC binding by POB only at concentrations below 4 nM ^{3}H-DHEC cannot be readily explained. Finally, our previous paper was misquoted. Attention to the reported data would indicate that oestrogen increased ^{3}H-DHEC binding at all concentrations from 0.5 to 100 nM ^{3}H-DHEC[3].

JAMES M. ROBERTS
ALAN GOLDFIEN
PAUL A. INSEL

Department of Obstetrics, Gynecology and Reproductive Sciences, Medicine and the Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California 94143

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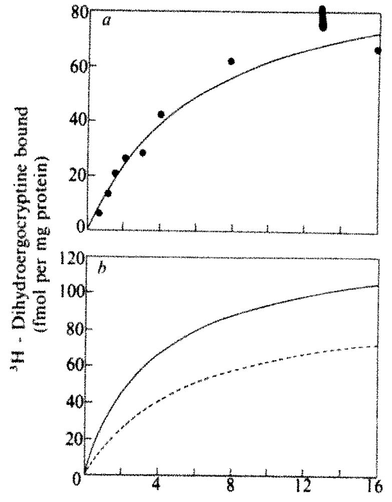

Fig. 1 *a*, Computer modelling of the control binding curve from Kunos *et al.* (Fig. 2, ref. 1). The solid circles represent graphical estimates of the binding data obtained by computerised digitisation of the published figure. The solid line is the best fit to a model for one class of binding sites according to the mass action law. The data could not be succesfully fitted to a two-site model. K_d = dissociation constant of receptors for ^{3}H-DHE = 6.2 ± 1.4 nM, R = receptor concentration = 101 ± 14 fmol per mg. *b*, Computer simulation of a control (solid line) and a POB-treatment (dashed line) binding curve, based on parameter estimates provided by Kunos *et al.*[1,2]; two classes of binding site (K_d = 1.5 nM and 5.5 nM, R = 37 and 97 fmol per mg protein) for the control curve and one class of binding site (K_d = 5.5 nM and 97 fmol per mg protein) for the POB-treatment curve (compare with Fig. 2, ref. 1).

THE report by Kunos *et al.*[1,2] on the characteristics of tritiated dihydroergocryptine (^{3}H-DHE) binding to rabbit uterine α-adrenergic receptors suggested that (1) DHE binds with differential affinity to two classes of α-adrenergic binding sites (K_d values of 1.5 and 5.5 nM, respectively); (2) phenoxybenzamine (POB), at a concentration which completely blocks the contractile response to the α-agonist noradrenaline, also selectively blocks the higher affinity class of α-adrenergic binding sites (those presumably labelled at DHE concentrations ≤3 nM) while having no effect on the lower affinity sites (those presumably labelled at DHE concentrations >3 nM).

The first conclusion which is seemingly at variance with published reports from several laboratories[3,4,5], is based on the observation of a break in the hand-drawn curve of a binding isotherm for ^{3}H-DHE (Fig. 2, ref. 1). We obtained graphical estimates of their binding data and subjected them to computer modelling, using well established methods[6–9]. Figure 1*a* (compare with Fig. 2, ref. 1) shows that their data were best fitted with a model for one class of DHE binding sites ($K_d = 6.2 \pm$ 1.4 nM, $R = 101 \pm 14$ fmol per mg protein). Attempts to fit the experimental data with a model for two classes of DHE binding sites were unsuccessful in providing parameter estimates, indicating that the data did not support the hypothesis of more than one class of DHE binding site. Computer modelling of the first five points (DHE ≤3 nM) of their binding isotherm provided similar parameter estimates for one class of DHE binding site although much less accurately ($K_d = 6 \pm 6$ nM, $R =$ 90 ± 60 fmol per mg), again in contrast with the results of their subjective graphical analysis of these early points. In agreement with the findings of other investigators[3–5] and in conflict with their own interpretation, the data of Kunos *et al.* actually indicate the presence of a single homogeneous population of DHE binding sites in rabbit uterine membranes.

Despite the inability of their experimental data to support objectively the hypothesis of two classes of DHE binding site, the parameter estimates reported by Kunos *et al.*[1,2] for these two putative classes of DHE binding site could be used to provide computer simulations (Fig. 1*b*) of control and POB-treatment curves. Such simulations should reproduce the salient features of the data as interpreted by Kunos *et al.*—the presence of a break in the binding isotherms and a difference in the control and POB-treatment curves only at low DHE concentrations (DHE ≤ 3 nM). In contrast to the hand-drawn binding curves, the simulations revealed no break. The predicted POB-treatment curve was also noticeably lower than the control curve at all concentrations of DHE, as expected after blocking of the high affinity class of binding sites, again in contrast with their experimental data.

Their second conclusion also contrasts with previously published findings[4] which indicated that POB treatment only reduced the binding capacity of the uterine α-adrenergic receptors, leaving unchanged their uniform affinity for DHE. It has been established by several laboratories that at least two distinct types of α-adrenergic receptor, termed α_1 and α_2, can be identified in radioligand binding studies[9–12] and physiological studies[13]. DHE has been found to bind with indistinguishable affinity to both subtypes of receptor[3,4,9,10]. By contrast, phenoxybenzamine has been shown in some physiological studies to be α_1 selective[13]. Thus, the observation by Kunos *et al.*[1,2] of complete blocking of the α-agonist response in the face of partial loss of DHE binding (Fig. 1, ref. 1) may be interpreted as the result of selective blockade of the α_1 subtype of DHE binding site. However, this conclusion cannot be substantiated by the DHE binding data that they provided (Fig. 2, ref. 1) and would require more quantitative binding studies involving α_1- and α_2-selective ligands such as prazosin and yohimbine, as presented elsewhere[9].

In conclusion, inappropriate and erroneous interpretation of binding data have led Kunos *et al.*[1,2] to incorrect interpretations concerning DHE binding to uterine membrane preparations. In addition, the effect of POB treatment on DHE binding at higher ligand concentrations (8 nM) reported in Figs 1 and 2 (ref. 1) are internally inconsistent. All available data (including those of Kunos *et al.*) indicate that DHE binds to uterine α-adrenergic receptors all of which show indistinguishable affinity for DHE.

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ANDRE DE LEAN
ROBERT J. LEFKOWITZ

The Howard Hughes Medical Institute,
Departments of Medicine and Biochemistry,
Duke University Medical Center,
Durham, North Carolina 27710

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KUNOS *ET AL.* REPLY—In answer to the points raised by Roberts *et al.*, the definition of receptors involves a physiological function: our findings[1] dissociate the phentolamine-suppressible binding of high concentrations of dihydroergocryptine (DHEC) from α-receptors mediating smooth muscle contraction. The low potency of phenoxybenzamine (POB) in suppressing DHEC binding was evident in earlier studies[2]; our findings confirmed and explained this discrepancy. As for the specific points raised: To their first point, yes, we should assume that, in the demonstrated absence of 'spare' α-receptors in our system[1], the potency of POB should be the same for block and for suppression of binding. Efficiency of coupling does not affect antagonists unless they act at a stage subsequent to receptors. It is also erroneous to suggest that block of responses to noradrenaline involves non-adrenergic receptors. Even if low concentrations of POB interacted with other receptors, this would be irrelevant to its effect on DHEC binding; even so, we found that serotonin and pilocarpine have no mechanical effects in rabbit uterine strips, and contractions by histamine are unaffected by 3×10^{-8}M POB, which blocks noradrenaline completely.

Concerning their second and third points, K_d values were determined in individual preparations but, due to the limited amount of membrane material, data points were concentrated either below or above 3 nM. If POB treatment is omitted, more control points can be obtained. Recently, we confirmed a small break in the DHEC binding isotherm constructed from 18 data points at close concentration intervals. Scatchard plots of such curves are linear points below 3 nM and curvilinear with initial upward convexity above 3 nM; this is very similar to recent findings in the rat heart[3]. In this latter study, positive cooperativity between sites of the second binding component has been proposed to account for the curvilinear Scatchard plot. In the absence of sufficient numbers of closely spaced data points in the area of the break (2–4 nM) such deviations are difficult to identify, particularly if the linear sections of the plot have similar slopes. Also, we found that because DHEC is adsorbed to glass and many plastic materials, total ligand concentrations must be verified in aliquots of every assay tube to avoid errors in calculating bound/free values. In answer to the fourth point of Roberts *et al.*, the reason that an expected small suppression by POB at higher DHEC concentrations was not detected is not clear. It certainly could not be explained by reversal of the block by POB. The irreversible nature of the block of α-receptor responses by POB is generally accepted[4] and this was also shown for block of ^{3}H-DHEC binding to uterine membranes in conditions identical to those used by us[5]. Direct evidence for the irreversible binding of POB to α-receptors was recently provided in studies using ^{3}H-POB of high specific activity in intact liver cells[6] or liver membranes of rats[7]. It is possible that the expected 20–30% suppression of binding may have been obscured by the relatively large variation of values at higher DHEC concentrations (Fig. 2, ref. 1). In a different set of experiments, shown in Fig. 1, ref, 1, the same POB concentration (3×10^{-8} M) suppressed the binding of 8 nM ^{3}H-DHEC by 27%. Finally, the ligand concentrations are not easily identifiable from the Scatchard plots shown[8]: in oestrogen-treated preparations there seem to be no more than three points or less than one point per preparation under 3 nM ^{3}H-DHEC.

The points raised by De Lean and Lefkowitz are the same as the third and fourth points of Roberts *et al.*, but they are also illustrated by computer analysis of our data. The inability of the computer to detect a break in our control binding curve is not unexpected; the k_d values for the two binding components were very close[1] and as the K_d of the second binding component was determined from points outside the area of the break (see Fig. 2 legend[1]), possible localised changes in binding affinity would not be reflected in the value obtained (see above). It is clear that on the basis of these data alone we should have disregarded the small break in our control curve, which is similar to the break evident in the DHEC binding isotherm reported by Williams *et al.* (Fig. 2, ref. 2). However, we could not have disregarded the major difference in the ability of POB to block DHEC binding below and above the same concentration where the break in the control curve is apparent (Fig. 2, ref. 1). Such a differential effect of a suppressing ligand is only possible if the POB-sensitive and POB-insensitive sites have different DHEC binding properties. Failure of the computer to simulate our sigmoidal POB curve indicates that DHEC binding in the real tissue is more complex than the model on which the computer program is based. For example, positive cooperativity between sites of the second binding component is a possibility compatible with all our findings (see above), although a detailed analysis of the second binding component at different levels of receptor occupancy[3] was not possible due to the limited number of data points.

In agreement with our findings and in contrast to the above conclusions of De Lean and Lefkowitz, evidence in two recent studies also indicates binding of DHEC to two sites with differential affinity. In the rat heart ARC 239, an α-receptor antagonist similar to POB in its postsynaptic selectivity[3] was also similar to POB in preferentially suppressing the binding of low concentrations of ^{3}H-DHEC[3]. In the rat liver, dissociation of 20 nM ^{3}H-DHEC had two components, indicating two binding sites with different affinities[9].

Thus, our results[1] indicate the presence in the uterus of two DHEC binding sites, only one of which, preferentially labelled at concentrations <3 nM, is involved in α-receptor mediated smooth muscle contraction. The exact nature of binding of DHEC to the second class of binding sites and the physiological function of these sites in the uterus remain to be clarified. Computer analysis of binding isotherms will give rise to erroneous conclusions unless the validity of the model used is firmly established.

GEORGE KUNOS
YIN NAM KWOK
WAI HO KAN
LUCIA MUCCI

Department of Pharmacology and Therapeutics,
McGill University, Montreal, Quebec, Canada

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Sensitivity of human bone marrow cells to cyclosporin A

FROM observations of the effect of cyclosporin A *in vitro* on committed progenitor cells of the myeloid and erythroid series (CFU-C and BFU-E), Gordon and Singer[1] have inferred that cyclosporin A has non-myelotoxic immunosuppressive activity. We find this a disturbing conclusion because although similar studies in this laboratory[2] using the semi-solid agar CFU-C assay[3] showed almost identical dose-related inhibition of CFU-C, our results also suggested that sensitivity to cyclosporin A is related to the level of differentiation of progenitors in the granulocyte–monocyte pathway, the more primitive progenitors being more sensitive. This is important because the pluripotent haematopoietic stem cell (CFU-S) from which CFU-C are derived and on which haematopoietic recovery in marrow transplantation depends, may be more sensitive to cyclosporin A than results of CFU-C assays indicate.

There is now considerable evidence that clone size potential decreases and sensitivity to factors with colony-stimulating activity (CSA) increases as progenitor cells differentiate down the granulocyte–monocyte pathway[4,5].

Our experiments showed that: (1) cells forming small clones were less sensitive to cyclosporin A than cells forming large clones; (2) cells requiring the highest levels of CSA for clone formation (that is, the most primitive progenitors) were more sensitive than cells forming clones at lower CSA levels.

Both observations point to a trend of increasing sensitivity to cyclosporin A as the CFU-C/CFU-S transition is approached and thus to the possibility of considerable myelotoxicity in conditions where CFU-S are rapidly proliferating. Even without speculating on the sensitivity of CFU-S, our results for CFU-C detected at the highest CSA levels (approximately 50, 70 and 90% inhibition at 1, 5 and 10 μg ml^{-1} of cyclosporin A, respectively) are disturbing, as 5–10 μg ml^{-1} was required for near-maximal inhibition of the most sensitive T-lymphocyte colony-forming cell population in two of three patients studied by Gordon and Singer[1].

G. E. FRANCIS
J. J. BERNEY

Department of Haematology,
The Royal Free Hospital,
Hampstead, London NW3, UK

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GORDON AND SINGER REPLY—The point that cyclosporin A may be considered to have some myelotoxicity *in vitro* is well taken. In terms of bone marrow engraftment, however, myelotoxicity has not been a problem. Cyclosporin A increases the survival of rabbits grafted with allogeneic marrow[1] and is an effective prophylaxis against graft-versus-host disease in man[2,3].

Francis and Berney have suggested that pluripotential stem cells (CFU-S) may be more sensitive to the compound than committed progenitor cells (CFU-G) and that cyclosporin A may have greater toxicity towards rapidly proliferating cells. These suggestions are based on the findings that small clones, grown in agar culture, are less sensitive to cyclosporin A than large clones and that sensitivity to the compound increases with the amount of colony-stimulating activity (CSA) presented to the treated cells.

Our data[4] do not show whether cycling and non-cycling haematopoietic progenitor cells differ in their sensitivities to cyclosporin A as the study used normal steady-state cells. This aspect is certainly of interest and experimental evidence can be obtained by studying the effects of cyclosporin A on patients' colony-forming cells following bone marrow transplantation.

The design of the experiments[5] said to show an effect of CSA activity on cell sensitivity to cyclosporin A involves a preincubation step before the cells are washed and plated in agar. It seems reasonable to suggest that the apparent effect of CSA activity on cell sensitivity may result from an altered responsiveness of the cyclosporin A-treated cells to CSA (or to inhibitors). Again, this can be tested by producing full dose–response curves of post-cyclosporin A cells, preferably to a cell-free source of CSA, which will give more reliable results than cellular feeder layers.

M. Y. GORDON

Department of Haematology,
Royal Postgraduate Medical School,
Hammersmith Hospital, London, UK

J. W. SINGER

Veterans Administration Hospital,
Seattle, Washington

1. Jacobs, P. *et al. Int. Soc. Haemat. II* (abstr.) 17 (1979).
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Correlation between heart attacks and magnetic activity—a retraction

WE recently showed a significant correlation between the daily sums of Kp, the planetary index of geomagnetic activity, and the daily admissions of cardiac emergency cases to the two main hospitals in Hyderabad and Secunderabad[1]. Richard Peto of the Radcliffe Infirmary, Oxford, has re-examined our data, and finds that their distribution about the regression line is grossly sub-poissonian. This means that the correlation is higher than would be expected even if magnetic activity were the sole cause of heart attacks, and casts serious doubt on the authenticity of the data. The magnetic activity data are those published by the International Association of Geomagnetism and Aeronomy, and are above suspicion. The medical data are those used by Srivastava, Bhaskara Rao and Saxena[2] and were abstracted by colleagues of Srivastava (Bhaskara Rao, Saxena and two others) from individual case sheets and the hospital records. Srivastava and Saxena have re-examined the hospital records, which are now in a more accessible form, and are unable to reproduce the numbers abstracted earlier. We conclude that the medical data used in the two studies[1,2] are spurious, and any results derived from them should be disregarded. This may explain why Knox *et al.* have carried out a similar enquiry and failed to confirm our claims[3].

We are grateful to Dr Peto for drawing our attention to this matter, and apologise for publishing a misleading result.

S. R. C. MALIN

Institute of Geological Sciences,
Murchison House,
West Mains Road, Edinburgh, UK

B. J. SRIVASTAVA

National Geophysical Research Institute,
Uppal Road, Hyderabad 500 007, India

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Glaciation due to continental drift

COGLEY[1] has suggested that a special distribution of land is needed during glacial periods. He proposes that the onset of glaciation occurs when land masses congregate in the tropics. The average global temperature decreases as the polar regions become "slightly warmer while

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the tropics cool substantially". This is a consequence of the albedo contrast between land and water varying with latitude.

The physical situation is more complicated than Cogley suggests and it is possible that many mechanisms operate which can induce glaciation. Tarling reported an analysis of ice ages which suggested that a continental block is needed in polar areas[2], especially in the Southern Hemisphere. Nevertheless, a near-polar position is insufficient to induce glaciation[3], a point with which Cogley concurs. But his argument depends on variations in albedo contrast. Tarling maintains that adjacent seas are necessary to provide moisture for precipitation. In such conditions the mid-latitudes become sensitive to comparatively small changes in radiation such as those caused by variations in the Earth's orbit and the tilt of its axis[2]. However, contemporary theories of glaciation involve galactic mechanisms[4–6] only insofar as they influence the intensity of solar radiation. In what way can the reduction of solar radiation due to the Solar System passing through a dense galactic cloud (which has been shown to be related to the occurrence of ice epochs[4]) "modulate known tectonic mechanisms" here on Earth?

The distribution of the continents is evidently important. Oceanic current flow, cloud cover and atmospheric circulation, which ultimately determine the amount of heat absorbed, are all affected by continental drift. In the late Cenozoic era, Australia and Antarctica drifted apart, commencing around 55 Myr BP[3]. Circum-Antarctic oceanic circulation increased as the continental barrier was gradually removed with deep water flow occurring after the South Tasman Rise had subsided around 38 Myr BP[3]. After this time an increase in the extent of pack ice occurred with a profound effect on the Southern Ocean[3] and on polar albedo. Kennett also reports that equatorial oceanic circulation diminished during the Cenozoic because of the destruction of the Tethian Seaway and, slightly later, the blocking of the Indonesian Seaway.

On what grounds does Cogley assume that sea level has remained constant between 240 Myr and 0 Myr? Eustatic changes of up to several hundred metres have been proposed[7–9]. A quantitative assessment of changes of land area with latitude and with time cannot be made from maps of palaeocontinents delimited only by the present day shoreline[10]. For example, land areas on the North American continent in the late Cretaceous were at least 50% less than at present[9], a fact that cannot be derived from palaeocontinental maps which do not show any variation of coastline with time. Using Cogley's own formula (equation 1) the change in land area of North America alone would produce an increase in the average global absorbed radiation (about $1\,\mathrm{W\,m^{-2}}$) which is significantly greater than the variations he proposes through continental drift. Cogley states that the Mesozoic was warm, yet he presents a diagram of average global absorbed radiation over 240 Myr with a minimum (implying coolness) during the Mesozoic. If land area changes are taken into consideration, there should have been an increase in absorbed radiation.

Cogley should have considered the effect of clouds in more detail. Cloud plays a very important part in the global radiation balance[11] and determines the atmospheric transparency term in his equation 1. He has argued a dynamic model of glaciation from static components: from a snow-free world with contemporary cloud cover, from a world without changes of sea level and without pack ice. Albedo contrast may well play a part in initiating glaciation but the evidence cited does not support Cogley's thesis.

JOHN M. REYNOLDS

British Antarctic Survey,
Natural Environment Research Council,
Madingley Road, Cambridge, UK

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COGLEY REPLIES—I take Reynolds' most important point, that my model is simple while the world is complicated. I do not claim that the model is a comprehensive theory of glaciation; I say merely that physical reasoning leads us to expect a palaeoclimatic signal due to albedo contrast, and that if we could find it this signal would deepen our understanding of glaciation. Reynolds is entitled to reject my interpretation of Fig. 1*c* of my letter. He seems to have no quarrel with the concept of albedo contrast, but to claim instead that the signal is likely to be swamped by other effects. This would not surprise me in the least, and I look forward to accurate estimates of these effects.

Matters Arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature.* The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

Many palaeogeographic effects are consistent with my model. A tropical clustering of landmasses surely suggests reduced tropical, and enhanced higher-latitude, meridional circulation in the oceans[1]. Kennett's evidence for sea ice, found in Southern Ocean sediments after 38 Myr BP, is not in conflict with what I deduced from the concept of albedo contrast. Note that I made no attempt to model the transition to glacial conditions; by design, my radiative calculations were for a snow-free world without pack ice, a state of affairs for which the apparently almost complete absence of glaciogenic rocks in the Mesozoic[2,3] provides circumstantial evidence.

I am unable to comment further on galactic mechanisms; my use of the verb 'modulate' was unthinking.

I assumed constant sea level because I wished to proceed from the simple to the complex. Maps of ancient shorelines carry uncertainties of their own[4], and the only global set[5] is 30 years old; although it was carefully compiled, it needs updating. One should be clear as to how continental flooding bears on my model. To nullify the concept of albedo contrast, flooding would have to be strongly biased towards either high or low latitudes, but such a bias is not seen. I fully agree that flooding may be important in its own right, and will affect my numerical results; I have indeed repeated the procedures of my letter for a series of equal-area maps based on the Termier and Termier set[5], but I am not yet able to discuss these results.

I agree also that clouds deserve thoughtful attention, but I emphasise again that my extremely simple model remains physically sound. I suggest that the time is ripe for modelling Phanerozoic climates at finer scales of resolution, and in increasing detail. However, I would caution that efforts at improving accuracy[6] should keep pace with speculative insight.

J. GRAHAM COGLEY

Geography Department,
Trent University,
Peterborough, Ontario, Canada K9J 7B8

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BOOK REVIEWS

Changing views of man

Antony Flew

Scientific Models and Man: The Herbert Spencer Lectures, 1976. Edited by Henry Harris. Pp.102. (Oxford University Press/ Clarendon: Oxford, 1979.) £5.95.

THIS slim volume consists of five of the six 1976 Herbert Spencer Lectures, a paragraph of Preface, and an Index. There are five only because the sixth lecturer, whom the Editor does not scruple to name, produced no script. No information about the contributors is provided, and there are more misprints — none of these sense-destroying — than one expects under the Oxford imprint. The Editor does not quote the terms of reference under which the lecturers worked. But, speaking presumably for the electors, he does say: ". . . we have gathered together a group of distinguished men from different disciplines to tell us how current work in their own subjects is changing their views of man".

The first essay, by Amartya Sen, is "Rational fools: a critique of the behavioural foundations of economic theory". The target in Sen's sights is that theoretical fiction, economic man; who is, by definition, "actuated only by self-interest" (page 1). But he seems not fully to appreciate how drastically this fiction is itself changed if you then proceed "to define a person's interests in such a way that no matter what he does he can be seen to be furthering his own interests in every isolated act of choice" (page 5). For a person who is thus necessarily in every choice striving — as the economists say — to maximise his own utilities may well be — in the everyday sense — acting very disinterestedly. What Sen explores is the huge area in which actual behaviour, though necessarily maximising the agent's utilities, is by no means reducible to the pure pursuit of that agent's self interest, as ordinarily construed.

In "Psychology and the image of man", Jerome Bruner confesses that in working on his lecture he was "drawn to a disturbing conclusion on the matter of why experimental or academic psychology had not had more of an impact on the broad cultural conception of the nature of man . . ." (page 27). I now confess that, having read the resulting lecture, I am more than ever inclined to think that the reason is that it has had nothing to say that should be of general interest: the genuine discoveries have no wider implications; while the claims which do have a shattering impact are not supported by the empirical work. Skinner, for instance, denies the reality of human choice and human purpose. But these claims are what he mistakes to be presuppositions of any science of psychology. They neither are nor could be findings of his own studies of rats and pigeons.

In "Memory and its models" J.Z. Young writes: "The concept that the brain contains information in a code is the new scientific model of memory, which I believe is revolutionising our view of man" (page 45). I am thus startled to be made to feel that I have all my life been a child of the revolution. For I cannot recall ever believing that information is stored in the brain in the form in which it is remembered. How could it be?

"Machine models of perceptual and intellectual skills" by Donald McKie is fascinating stuff: "The world has a false belief about calculating prodigies, namely that they calculate prodigiously" (page 74). The truth is, apparently, that they use all manner of calculation-saving dodges. The same is true of chess-masters: they neither do nor could work through every thinkable alternative many moves ahead. To achieve a Grandmaster level of play by a computer program on these lines: "It would . . . take of the order of 10^{90} years' continuous running on a super-fast machine to select a move" (page 77). For the benefit of possible philosopher-readers of *Nature* I have to mention the suggestive treatment of the seventeenth-century Problem of Dr Molyneux: "R.L. Gregory studied just such a case, in which the patient had had sight conferred on him by an operation of corneal grafting" (page 62).

In excellent taste, the most exciting contribution is kept till last. In "The universe as artefact", D.H. Wilkinson ponders Einstein's homespun question: "What does a fish know about the water in which he swims all his life?" (page 80). Wilkinson's ponderings raise questions about ways in which the basic facts about our nature — size, length of life-cycle, and so on — must eventually limit our possible physical knowledge: ". . . already in the big sciences it takes typically 5 years from inception to completion of an experiment . . . When we get to 100-year long experiments which have to be passed from academic father to academic son we shall not have many takers . . ." (page 82). But I wish that Wilkinson had ended, not by first mentioning and then repudiating Berkeleyan idealism, but with his earlier words: "'Perhaps the impossibility of finding quarks is Nature's way of letting us know that we have reached the end of the line.' But we know that we could not accept that." (page 97). □

Antony Flew is Professor of Philosophy in the University of Reading, UK.

Crossbridge theory in the dock

R.M. Simmons

The Molecular Basis of Force Development in Muscle. Edited by N.B. Ingels. Pp. 162. (Palo Alto Medical Research Foundation: Palo Alto, California, 1979.) $17.

THE sliding filament theory of muscular contraction is generally accepted as well established. Less certain is the mechanism of force generation and shortening, but the great majority of workers in the field accept some form of crossbridge mechanism at the very least as a working hypothesis. There are dissidents from the majority view. This book, the proceedings of a conference held in Miami in 1977, is edited by one of them and the conference evidently served as a platform for the views of another (Pollack). Perhaps court-room would be more appropriate than platform, for Pollack seizes the roles of prosecutor, judge, jury and would-be executioner, with orthodoxy embodied by H.E. Huxley, Podolsky, Weber and Julian in the dock.

The trial opens with short pleas from the defendants. The case is nicely argued on the grounds of structure (Huxley and Podolsky), biochemistry (Weber) and mechanics (Julian and Podolsky). The prosecution case from Pollack, subsequently enlarged on in a lengthy cross-examination of the defendants, involves two main charges. The first is that the constancy of filament length demanded by the sliding filament theory is not always to be found in the literature, but Pollack's attempt to weigh the evidence by number of pages rather than by scientific merit becomes more of a trial of patience than of fact. Huxley defends the case as trenchantly as he did twenty years ago and the theory emerges from the trial without much of a stain on its character. The second charge concerns the length-tension relationship, one of the corner-stones of the crossbridge hypothesis. Gordon, A.F. Huxley and Julian showed in 1966 that the tension developed by a muscle fibre depends linearly on the overlap between the filament, though if stimulation of stretched fibres is prolonged tension continues to rise slowly. This was ascribed to the shortening of some of the sarcomeres. Pollack states that he does not observe such a shortening, at least using light diffraction. On the other hand Julian says he does, using photography. A fair verdict would be that the charge is "not proven."

Ingels makes what seems to be his second contribution to the field with a chapter on "Unifying Concepts" which attempts to unify his first contribution, a non-crossbridge theory, to crossbridge theory, but little else. As organiser of the conference he must bear some responsibility for the fact that nearly two-thirds of the book is unrepresentative of current research in the field. The chapters on crossbridge theory and practice might serve as an introduction to the field and contain some new results particularly from X-ray diffraction studies, but they are too brief. The discussion never gets away from the two issues reported above. The question of whether current results from structural, biochemical and mechanical studies can be explained on other than a crossbridge model is not raised. There is an Appendix from Ingels with a chronology of muscle theories, some of which are splendidly batty. □

R.M. Simmons is Lecturer in Physiology at University College, London, UK.

Molecular basis of tumourigenicity

Dennis D. Cunningham

Surfaces of Normal and Malignant Cells. Edited By R.O. Hynes. Pp.471. (Wiley: Chichester, UK, and New York, 1979.) £24.

THE cell surface has become a prime target for studies on the molecular basis of tumourigenicity. In a lucid introductory chapter, the editor summarises reasons for this, discusses issues basic to such studies (for example, do properties of transformed cells in culture accurately reflect the properties of malignant cells *in vivo*?), and sets the stage for the subsequent contributions.

A chapter on morphology of normal and transformed cells is followed by three chapters on cell surface composition which emphasise glycolipids, proteins and glycoproteins, and mucopolysaccharides. Two chapters focus on enzymes of normal and transformed cells, and there is a chapter on immune responses to C-type virus-induced tumours. From here on, the chapters mostly deal with control processes in normal cells: early events in growth stimulation, surface transport components of erythrocytes, adhesive specificity among embryonic cells, and the cell surface and development in the cellular slime mould *Dictyostelium discoideum*.

A theme which recurs in this volume is that the several properties which appear to correlate generally with the transformed state are not invariably found in all transformed or tumourigenic cells; it is probably certain combinations of these, along with alterations yet to be defined, that collectively underlie malignancy. Given present uncertainties about which of the many alterations found in transformed cells and their surfaces might be causally related to malignancy, and the fact that any given topic on regulation of cellular activities could relate to alterations basic to malignancy, the editor faced a real challenge when choosing the issues to be addressed in this volume.

Although most of the choices were excellent, it would have been an easy matter to justify individual chapters on assays for tumourigenicity (malignancy?) of cultured cells, and the use of melanoma lines with specific metastatic potentials to probe cell surface properties related to metastasis. As emphasised by the editor, the commonly used assays for tumourigenicity of cultured cells depend on growth and survival of the cells and not generally on their ability to invade and metastasise, even though the latter two properties distinguish malignant tumours from ones that are benign.

As "a mere collection of data would soon become out of date . . . the authors were encouraged to extrapolate from the established facts and discuss hypotheses concerning their significance". As a result, the individual chapters are generally conceptual in nature, and provide the reader with interpretations of results. Thus, the volume does what this type of book should: synthesise and extrapolate rather than present previously published data. At the same time, each chapter is quite well referenced, providing the reader with ready access to the original literature. In general, the individual chapters are well written and the authors emphasise questions that remain to be answered. Two chapters, "Surface Membrane Enzymes in Neoplasia" and "The Cell Surface and Development in the Cellular Slime Mould *Dictyostelium discoideum*", were disappointing. Both were rather long on facts and short on interpretation. Unfortunately, the latter chapter did not contain a much needed summary section.

My overall impression of this book is quite favourable. It generally emphasises ideas and questions; at the same time, it contains enough experimental facts to be informative and credible. With exceptions in only several parts, the book is well integrated, a feature which should increase its general appeal. □

Dennis D. Cunningham is Professor of Microbiology at the University of California at Irvine.

Wave functions for atoms and molecules

A.D. Buckingham

The Calculation of Molecular Orbitals. By J.C. Slater. Pp. 104. (Wiley: New York and Chichester, UK, 1979.) £11.50; $23.

PROFESSOR J.C. Slater was a theoretician of the first rank and a prodigious writer of textbooks. The present work is the last in a series of eight books by Slater on the quantum theory of matter (the first was published in 1960). The manuscript of this book was found among his papers following his death in 1976, the only addition being a list of a small number of references.

In the last ten years of his life, Slater was concerned with the local-density form of the self-consistent field; his work led to the so-called $X\alpha$ method, and he and many others applied it to a wide range of problems in theoretical physics and chemistry.

This new book of only a hundred pages centres on the SCF $X\alpha$ method for obtaining approximate electronic wave-functions for atoms and molecules.

Applications of the $X\alpha$ method have involved approximations which impose limitations on the validity of the results. Slater's purpose in writing this book was to suggest a different scheme for solving the SCF equations. He turned to the cellular method, developed by Wigner and Seitz for handling the problem of computing the orbitals of a crystal. (Slater himself was a pioneer in this energy-band theory.)

All those who are interested in the SCF $X\alpha$ method will find this a most useful and informative book. It is the writing of a mature scientist, one of the masters of theoretical physics and chemistry. □

A.D. Buckingham is Professor of Chemistry at the University of Cambridge, UK.

Single-stranded DNA phages

Mary R. Lunt

The Single-Stranded DNA Phages. Edited by D.T. Denhardt, D. Dressler and D.S. Ray. Pp.720. (Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1979.) $49.

IT is now twenty years since Sinsheimer wrote that finding a single-stranded DNA molecule was "like encountering a unicorn in the ruminant section of the zoo". Since then studies on the single-stranded DNA phages have not only produced information relevant to fundamental biological processes but have contributed much to the development of methods now routinely applied in protein and nucleic acid biochemistry.

This book arose from an international meeting held in August 1977, but includes references to papers and unpublished work up to 1978. The articles are comprehensive and well-written reviews of all aspects of the biology of the single-stranded DNA phages. They include discussions of the structural and regulatory roles of the protein-nucleic acid interactions involved, thus providing interest well beyond the specialist areas considered.

The papers mainly refer to the isometric phages ϕX174, S13 and G4, and to the male-specific filamentous phages f1, fd and M13. They all use *Escherichia coli* as host and have virions which contain single-stranded, covalently closed, circular DNA of molecular weight about 2×10^6 and coding for nine genes. Besides being the first virus shown to contain such DNA, ϕX provided the first example of overlapping genes, that is, regions in which the DNA codes for more than one protein, the RNA transcripts being read in different phases. There are articles giving the complete nucleotide sequences of ϕX, G4 and fd DNAs, and discussions of the gene assignments and gene functions. The filamentous phages do not have overlapping genes, and can package variable amounts of DNA, as described in the use of M13 as a cloning vector.

The chapters on DNA synthesis review the many elegant experiments on the structures of the replicating forms, and their reproduction *in vitro*. The general significance of these studies is apparent in their analysis of the probable roles of host proteins in bacterial DNA synthesis, and also in their revelation of the importance of DNA secondary structure and its modification by enzymes such as DNA gyrase, helicases and the *E. coli rep* protein.

The analysis of transcriptional control of protein synthesis by these phages has revealed their ability to make a variety of polycistronic RNAs in which sequences coding for proteins made in large amounts appear in more than one RNA species. There are discussions of the structures recognised by RNA polymerase at initiation and termination sites, now made possible by the availability of DNA sequence data.

Increasing attention has recently been given to the formation of intact virions. The isolation of ϕX precursor particles is described and the protein-DNA interactions are discussed. The intriguing production of filamentous phage particles without cell lysis now seems to present an example of the 'signal' mechanism, as nascent coat protein molecules are probably embedded in the cell membrane by way of a leader sequence, the final protein product exchanging with the intracellular DNA binding protein as the phage DNA is extruded from the cell.

The chapters on filamentous phage virion structure provide detailed discussions of diffraction and other data which support different models for the spiral arrangement of protein monomers around the central DNA. There are also accounts of the use of single-stranded DNA phages in the analysis of genetic recombination, and of the disruption of virion structure which occurs when ϕX particles adsorb to the host cell.

The book is not for beginners, but it contains much which can be appreciated and enjoyed by advanced undergraduates and those who teach them. □

Mary R. Lunt is Lecturer in Biochemistry at the University of Oxford, UK.

Vitamin D metabolism and function

R.H. Wasserman

Vitamin D. By H.F. DeLuca. Pp.80. (Springer: Berlin, New York and Heidelberg, 1979.) DM29; $15.

VITAMIN D has long been appreciated as the primary factor required for the prevention and cure of rickets and osteomalacia in man and animals. This factor, acquired exogenously or synthesised endogenously by the skin upon exposure to UV light, undergoes biochemical transformations to yield various metabolites, including those hydroxylated in the 25 position [25(OH)D], in the 1 and 25 positions [e,25(OH)$_2$D] or in the 24 and 25 positions [24,25(OH)$_2$D]. The 1,25(OH)D has recently become recognised as a bona fide hormone since it affects reactions elsewhere than at the site of synthsis (kidney) and is under feedback control.

The biological effects of vitamin D and its metabolites have been extensively studied in recent years, and a considerable amount of new information and concepts on this aspect have also become available recently. Among these are the protein synthetic theory of vitamin D action, the identification of several proteins induced or modified by steroid repletion, the interaction of metabolites with the intestine, kidney and bone, and with organs not traditionally considered to be targets, such as the parathyroid gland and pancreas, and details on the translocation of calcium and phosphate across epithelial membranes, to mention just a few.

H.F. DeLuca has gathered together and integrated in a short monograph much of the recent advances. Specifically discussed in this volume are historical aspects, the chemistry of precursors and metabolites of vitamin D, the biological control of the formation of vitamin D by nutritional and hormonal variables, the molecular events affected by vitamin D that bear on calcium and phosphate metabolism, and therapeutic experiences with vitamin D and its metabolites.

Of considerable value are the critical analyses by the author of present concepts and of various published studies. Areas in which there is a paucity of information, and where controversy abounds, are frequently noted. However, not all experts in the field will agree with some of the interpretations and viewpoints of the author, perhaps an expected spin-off of a very active research area. For this reason, the reader is urged to consult other recent reviews and monographs on this topic in order to obtain a broader perspective.

The monograph is well written, lists about 400 references, and is reasonably well illustrated. Since the author and his associates around the world have been prolific contributors to vitamin D research, this monograph in a sense represents a cataloguing of their many contributions (about 30% of the references cited are authored or co-authored by Professor DeLuca).

This monograph should appeal both to the specialist on vitamin D, calcium and phosphorus metabolism, and to the interested non-specialist. It should also prove useful to the clinician, providing an introduction to the chemical and biological behaviour of a class of compounds that are efficacious in some disease states and potentially therapeutic in others. □

R. H. Wasserman is a Professor in the Department of Physical Biology and Section of Physiology, New York State College of Veterinary Medicine, Cornell University, Ithaca, New York.

Basic biology of *Drosophila*

Peter Lawrence

The Genetics and Biology of Drosophila. Vols 2a, 2b and 2c. Edited by M. Ashburner and T.R.F. Wright. (Academic: London, New York and San Francisco, 1979.) Vol. 2a: £28.50, $59; Vol. 2b: £29.60, $61.25. Vol. 2c: £32, $74.

ASHBURNER and Wright is a mammoth read. Or is it? This is the question for the *Drosophila* worker: Are the three parts of this volume a handbook for reference or a collection of ephemeral reviews for reading? If the former then the Drosophilist will want to buy them (or, rather, prefer to beg or steal them as they cost nearly £90). If the latter, then a lending library can be relied on and the relevant chapters read. It is not clear what the editors would have their books be. They write that it is an attempt "to gather together as much information on the basic biology of *Drosophila* as possible excluding only its formal genetics found in volume 1 and its population biology to be covered in volume 3".

I think these books deserve to be judged by high standards and to me that means as a handbook, a book which no *Drosophila* worker can be without because it contains the basic facts and methods. A handbook should make accessible the present state of hard knowledge, where the literature is critically distilled and not merely catalogued. This is especially true with *Drosophila* where an indexed bibliography is available. Just as important, the methods, hints, tricks and ploys should be given special emphasis. In brief, the chapters of a handbook should be written with the readers' needs in mind. By contrast, in reviews, the author is frequently thinking of making some kind of a sale.

As these three books have, necessarily, been written by many authors, it is no surprise that this purpose is not achieved by all, or even by most. Some chapters are, and will be, extremely useful — others are already out of date or just forgettable. The useful or essential chapters are mixed up with the relatively useless ones; the topics are also randomly assorted. This means, of course, that we have to buy each volume even if we need only a small part of each. For example, to obtain the critical review on chromosome puffing the cytologist has to pay for what is in effect a 150 page monograph on behaviour — this topic is unlikely to be close to his, or her, heart. One wonders whether this is one reason why big multiauthor books are becoming so popular with publishers — a small number of desirable pages can sell a larger number of undesirable ones. Always true of journals but not, in the good old days, true of books.

As I read through volume 2 it was not always easy to discern editorial purpose. The chapters are often of profligate and arbitrary length. Why, for example, should we have the same number of pages (100) on mutants which produce tumours (at the moment a somewhat unclear and peripheral subject for most Drosophilists) as on the central technical matter of chromosome aberrations (Volume 1)?. Why so much on the field of imaginal disc development and its genetics? This field is interesting, but it is moving just too fast to be reviewed at such length in a handbook. Thus, because of long publication delays, the chapters on this topic are already becoming obsolete (from the literature references I get the impression that they were written about 3 years ago). By contrast, the scanning electron microscope atlas of the fly will be useful to many and retain its usefulness for years. The collation of culture methods is admirable and here one can detect the special atmosphere of free exchange of know-how and stocks, which is such a pleasing aspect of work with the fruitfly.

In these books there are chapters on all manner of things: culture methods, both of flies and cells; nutrition; enzyme genetics; visual physiology; behaviour; tumours; chromosome puffing; the blood system; muscles; the fat body; malpighian tubules; ageing; embryonic development; imaginal discs and transdetermination. This means that every fly worker will want to read some parts and others will need to have the books within easy reach of their microscope. However, one cannot help feeling how much better they could have been. The chapters could have been more logically grouped. Ephemera could have been more conscientiously avoided. If the authors had been limited to numbers of pages which correlated with the importance of their subject, could this volume not have been at once shorter, cheaper and more valuable? □

Peter Lawrence is on the staff of the MRC Laboratory of Molecular Biology, Cambridge, UK.

Mechanisms of cell killing by radiation

J.H. Hendry

Cellular Radiobiology. By Tikvah Alper. Pp.320. (Cambridge University Press: Cambridge and New York, 1979.) Hardback £18; paperback £5.95.

THIS book would be more aptly titled '*Mechanisms in Cellular Radiobiology*' or more specifically '*Mechanisms of Cell Killing by Radiation*'. Written by one of the leading and often controversial figures in this field for a quarter of a century, the book traces in depth, both historically and scientifically, our knowledge of how various radiations cause a loss of reproductive integrity of cells when irradiated in different conditions.

It is the only book in recent years to compare and contrast fully and with clarity the response of different cell types from bacteria to mammalian cells, and it is this comparison which has provided much of our insight into the mechanisms of radiobiological effects. As the latter have not yet been fully elucidated, this has prompted hypotheses such as target theory and repair models to 'explain' dose–response relationships. These are discussed at length both in the first few and in the last few chapters. Sandwiched in between are the observations concerning dosage modifications caused by radiosensitisers (primarily oxygen), radioprotectors, ionisation density, phase of the cell cycle at which cells are irradiated, dose fractionation, and dose-rate effects.

The failure of target theory to accommodate certain critical radiobiological findings has led to the clear conversion of the author from target theory to repair-type models which are less rigid in their constraints. Perhaps as a consequence the correlation between chromosomal injury and cell lethality, now convincingly demonstrated, receives little attention. The treatise of repair-type or pool models is novel and comprehensive.

The book concludes with a chapter on measurements of cell survival *in vivo*, which is purposefully concise but which is consequently not as comprehensive as the other chapters. Also, in a few instances, effects are discussed which have not generally been found by other investigators, for example, aspects of survival and dose-rate effects in bone marrow.

Although there are many useful tables of collated data, this is clearly not simply a textbook for learning by rote. It is more a stimulating thesis to be digested, argued, even attacked, and will undoubtedly remain a firm favourite with mechanistic radiobiologists. In contrast to nearly all other specialised texts, the provision of a separate paperback edition puts this volume within everyone's pocket. □

J.H. Hendry is Head of the Department of Radiobiology in the Paterson Laboratories at the Christie Hospital and Holt Radium Institute, Manchester, UK.

OBITUARY

Peo Koller

P. C. Koller, Professor of Cytogenetics at the Institute of Cancer Research, died on 29 June 1979. He will always be remembered by those who knew him well. For some it will be his splendid humour that endeared him, for others his fascination for the facts of life.

Born in 1904, he was taken as a novitiate into a Benedictine community at the age of twelve with the intention that he should eventually become a teacher in a subject that he found pleasing. Choosing botany and zoology he eventually graduated from the University of Budapest in 1924 at the age of 20. During his time in the monastery and university he had had to combine religious and scientific studies and it is clear from what he wrote in his unfinished autobiography that there arose in his mind, even before he graduated, some difficulties of reconciliation of the two disciplines. Despite this he was ordained into the priesthood the same year he gained his BSc.

Rather to his surprise his superiors offered him a teaching post in Budapest and encouraged him to undertake a PhD. He met a number of scientists including Voronow, Rhoda Erdmann and Kammerer at an International Conference in Budapest in 1927 and became committed to a research career. Shortly after joining the new hydrobiology unit at Tihany on Lake Balaton he was granted a scholarship to go to Cambridge in the United Kingdom.

This was the beginning of his break with the church which was in a large part due to increasing interest in genetics. As he explained it, the determinist philosophy of genetics, with which he became involved under the influence of his collaborators T.H. Morgan, Theodore Dobzhansky, C.D. Darlington and H.J. Muller, was the deciding factor. His interests turned specifically to cytogenetics which was a newly emergent discipline in the early 1930's. During and immediately after the war he became involved with the effects of alkylating agents, such as nitrogen mustard, on chromosomes, an interest which remained for much of the rest of his working life. He joined the staff of the Royal Cancer Hospital and soon afterwards its pup, the Chester Beatty Research Institute.

Peo's study of chromosome breakage in tumours particularly under the influence of cancer chemotherapeutic agents established him as a leading authority in the field. At that time, however, further and more exact developments of cytogenetic studies of mammalian somatic chromosomes was somewhat hampered by the inadequacies of the available methods.

In 1956 under the influence of Alex Haddow he became interested in the newly discovered radiation chimaeras, the analysis of which was best accomplished by cytogenetic methods with which Peo was familiar and which, largely under the influence of Peo's friend, Charles Ford, were now more reliable.

Peo's curiosity led him with enthusiasm into the fields of haematology and immunology which emerged as integral parts of the study of chimaerism.

He was a great biologist and always had the ambition that biology should be accepted in its own right as a major discipline in cancer research. In his latter working years he provided encouragement for a number of young research workers including J.F.A.P. Miller who made his discovery of the function of the thymus whilst under Peo's umbrella.

He rarely scolded his juniors and always provided the quiet and unobtrusive support which is so successful and so difficult to emulate. It should not be thought that he lacked in passion. His occasional anger was a source of amazement and delight to his colleagues who, on one occasion at least, had to shelter from bombardment with the bottles in which *Drosophila* flies were kept.

Peo found great happiness in his marriage and his three daughters were a constant source of delight and pride in his life.

After retirement he became an articulate and much-respected member of the rural community in which he and his family chose to live. His preaching there of the delights and method of biological science was probably in many ways the happiest time of a very full life.

He will be remembered with affection and gratitude by his friends, his colleagues and his neighbours. There was rarely a dull moment with Peo about.

Tony Davies

C.E. Wynn-Williams

Dr Wynn-Williams, a pioneer in the use of radio valves, valve amplifiers and thyratrons for measurement and counting in the fields of nuclear and radiation physics, and in the basic techniques of electronic computers, died at his home near Aberystwyth on 30 August 1979, aged 76 years.

He was born in Swansea on 5 March 1903, attended Wrexham Grammar School and entered the University College of North Wales at Bangor in 1920 with an Open Scholarship and a County Exhibition. He received the R.A. Jones Mathematics prize in 1922 and graduated BSc Wales with first-class honours in physics in 1923. From 1923 to 1925 with the aid of a Research Studentship of the University of Wales he carried out research at Bangor under Prof. E. Taylor Jones, obtaining an MSc Wales in 1924.

He then continued with research in physics, at the Cavendish Laboratory, Cambridge, under Sir Ernest Rutherford, with, in succession, a University of Wales Open Fellowship, an 1851 Senior Fellowship and a moiety of the Clerk Maxwell Studentship. During this period he took a BSc London with first-class honours and received a Cambridge PhD in 1931.

His first two papers relating to valve amplification described a valve amplifier for ionisation currents with compensation for L.T. and H.T. battery fluctuations and equivalent to a quadrant electrometer of sensitivity more than 6000 mm per volt; and with the modifications of this necessary to allow it to be applied for measuring X-ray and photo-electric effects. He next worked in collaboration with Cave and Ward on the rate of emission of α-particles from radium, and with Rutherford and Ward on the α-rays from RaC, ThC and AcC, his main contribution being the design of the valve amplifier for the detection of the individual particles and its screening from outside effects.

A visit to the Cavendish Laboratory by A.W. Hull about the time of Hull's full description of thyratrons in 1929 stimulated Wynn-Williams' interest in them and led to his first step in their applications to counting when he devised a ring of four thyratrons and applied it in 1931 to further α-ray counting experiments with Rutherford and Lewis, in the investigation of long-range α-particles from ThC and AcC.

In the following year he published his "scale of two" thyratron counting system which, he pointed out, could be operated by current taken from the ordinary A.C. mains supply and required no delicate adjustments; it was this invention, followed six years later by a somewhat similar counter devised by W.B. Lewis emplying hard valves instead of thyratrons, from which has mushroomed the great technology of digital electronics. In recognition of this work Wynn-Williams was awarded the Duddell medal of the Institute of Physics in 1957.

During the second world war Wynn-Williams was deeply involved in the work of code-breaking machines, in particular on the "Enigma" cipher machine and on the electronic side of the "Heath Robinson" cryptanalytic device which was a step towards the later wholly successful "Colossus" machine. It is believed that he was at least in part responsible for the securing of T.H. Flowers and his team from the Post Office Engineering Research Station at Dollis Hill for the cipher work at Bletchley Park.

After the war, Wynn-Williams returned to Imperial College and threw himself whole-heartedly into teaching work in practical physics in which his great inventive skills and warm personality led to outstanding success.

The author of this obituary experienced these personal qualities to the full during the three years in which he was associated with Wynn-Williams at Cambridge and cannot speak too highly of him as a co-worker and friend, and for his help and encouragement.

F.A.B. Ward

Vladislav Kruta

MANY physiologists and historians of medicine in France, Great Britain, Czechoslovakia, and as far afield as New Zealand will be sorry to hear of the recent death of Professor Vladislav Kruta, MD, DrSC, past director of the Institute of Physiology of the Purkyně University in Brno, Czechoslovakia.

Born in 1908, his scientific career started in the early thirties at the Department of Physiology of Charles' University in Prague. From the beginning his interest was focused on the comparative physiology of heart muscle and on the effects of temperature on contractility. His research on the "Bowditch staircase" became a classic. His work brought him into close contact with French physiologists and especially with the school of Professor Lapique, where he spent a long period and where he also met his future wife. These contacts gave Czech physiologists access to the French and Western scientific forum: as a General Secretary of the Czechoslovak Biological Society he made this society a branch of the old and famous French Societé de Biologie.

As a convinced democrat, he left Czechoslovakia before the Second World War, spent a difficult period in France and was transferred after the fall of France with the Czechoslovak troops to Great Britain. As a medical officer, he became attached to the famous 312th Squadron of the Royal Air Force, composed mostly of Czechoslovak airmen, a squadron which won its glory during the Battle of Britain, with the loss of many lives. For his courageous conduct, Kruta was awarded one of the highest Czechoslovak military medals. Later in the war he was transferred to the Ministry of Social Affairs of the Czechoslovak Government in exile (in London) where he made use of his vast knowledge of the problems of nutrition to prepare the way in which the assistance of UNRRA should be made use of in his liberated home country. Czechoslovakia owes him gratitude for the generous contribution of UNRRA in reopening and restarting medical and biological research after the war.

In 1945, Kruta became one of the founders of the new medical school in the city of Hradec Králové. As with many other people who fought in the Allied forces in the West, he had been persecuted since 1948 and dismissed shortly afterwards from the Hradec Králové medical school, when it became a military medical academy under Russian supervision. He then took over the Chair of Physiology at the Purkyně University of Brno. In spite of many obstacles of a political nature he created there an excellent modern school of physiology, concentrating mostly on experimental cardiology.

His classical education and early interest in general history led him later to the history of medicine and mainly to the two great Czech protagonists of physiology — Procháska and Purkyně. It was Kruta's great achievement to continue and complete the edition of Purkyně's *Opera omnia*, started before the War by the Prague histologist Prof. Studnička, and to write a monograph on both these great figures.

During the Prague Spring in 1968, Kruta deeply engaged himself in the democratization of life in Czechoslovakia. He was one of the 70 original signatories of the manifesto of "2000 words" and after the Russian occupation, and in spite of pressure, he refused to recall his signature. This led to his immediate dismissal from the chair and from all academic activities. As a private person he continued his historical studies. His courageous and honourable life made him a lasting model to his compatriots and friends.

Jan Brod
Otokar Poupa

Robert Heizer

ROBERT FLEMMING HEIZER, who died on 16 July 1979, at the age of 64, was one of the best known and most outstanding archaeologists to have been trained and who taught at the University of California at Berkeley.

His contributions to his profession, to the scholarly world, to the public in general and to his University were continuous and at an extraordinary and prodigious rate and level of creative excellence that is rare, even in the most distinguished communities of scholars. He was the author of 24 books and nearly 400 scientific articles and monographs that express the remarkable breadth and wide-ranging interests of California's pioneer scientific archaeologist who believed that it is impossible to understand mankind or to formulate "laws" of culture without using the data of prehistorians.

Ranging from book-length treatments of the Olmec civilisation of Meso-America or the prehistoric rock art of California and Nevada (on which he was the acknowledged authority) to a study of prejudice and discrimination against Californian minorities under Spanish, Mexican and US regimes, or X-ray fluorescence analysis of obsidians and replicative experimentation in archaeology, his work also included excursions into such subjects as toxicology, geology, mineralogy, organic chemistry, pedology, demography, mathematics, architecture, history, limnology, law, conchology, zoology and economics, wherever he believed they could throw light on past human behaviour. His investigations at La Venta, not far from the Gulf of Mexico, as well as in the Valley of Mexico and Guatemala led him to develop a keen interest in the transport of megalithic monuments and the methods of erecting them which resulted in a brilliant piece of research on the origins of the Colossi of Memnon on the Nile.

The quantity and quality of his research and publication may in time tend to obscure his work and ability as a teacher. In this, however, he was equally successful and an inspiring lecturer at all levels of instruction. His teaching ranged from North American and Meso-American archaeology to California Indians, archaeology and society, and science in archaeology and he was equally as successful in field courses as he was in the classroom and in seminars. He was always available to talk with students, especially with any who came to discuss their original ideas.

Heizer's work is both the foundation on which systematic archaeology in California has been based and a contribution of major significance for the science of archaeology in general. This was recognised in the honours conferred on him: he was elected to the Society of Sigma Xi, was twice a Fellow of the Guggenheim Foundation, a Fellow at the Center for Advanced Study in the Behavioral Sciences at Stanford and was elected a member of the National Academy of Sciences in 1973. He received an honorary ScD from the University of Nevada in 1965.

Bob was the kind of scholar who will not be seen again for a long time. He was a great archaeologist to whom prehistory in the western states will forever be indebted. He was possessed of a rather rare sense of humour and could be extremely witty and good company. Critical of others, he was equally critical of himself and in some ways was a "loner". His friends remember him for his humour, kindness and generosity, his students for the way he shared with them the excitement of new discoveries in the field and colleagues and students alike for the magnitude and depth of his scholarship and for the stimulus that he gave to the discipline in general.

J. Desmond Clark

nature

10 January 1980

Biotechnology back in the limelight

A FEW years ago — in the mid to late 60s — there grew up a fashion for thinking that the 'new biology' would revolutionise the chemical and pharmaceutical companies. It has made its impact, but not so great as was first promised. Now again the genie rises, to face a number of problems — which we outline on pages 122-131.

The first flowering came with the elucidation of the sequences, structure, and function of a few enzymes, and their immobilisation to act as efficient, recoverable industrial catalysts, so that it became conceivable that all biology could be tamed and put to work. It affected mainly enzymologists and microbiologists. The second follows the discovery by molecular geneticists of techniques for manipulating and transferring DNA between organisms, and it has led to that new group of biologists discovering something quite different: the profit motive and the ways of industry.

Problems and acrimony have arisen between the biologists and industry, and among the biologists, particularly over the question of confidentiality. Scientists with one foot in the academic and one in the business world have felt a conflict between the need to publish fast and first in the former, and to keep secrets and respect patent law in the latter. In some of the smaller business ventures the urge to publish appears to have been effectively quashed; in others, it survives, if not completely intact.

Secrecy means that some academics cannot disclose to their colleagues the details of industry-funded research that is going on in the lab next door — souring relationships between them. For example the silence from most of the laboratories competing fiercely to sequence, clone, and manufacture interferon has, at times, been complete. But that is not surprising when what used to be an innocent amino-terminal acid sequence can be the key to a fortune.

These problems, though, should be temporary. They come about because the firms need to draw on a new community of scientists, one that is used only to the priorities of basic research. But already a new breed of molecular biologist is emerging which concerns itself not so much with understanding basic mechanisms as with manipulating them to reach desired goals. The joys of mission-oriented research are totally different from those of simple inquiry, and slowly they will sort out a different kind of biologist, and eventually a different career structure, different opportunities and relationships. It was in just this way that the devices engineer became separated from the solid state physicist, and the nuclear power specialist from the physicists who play with quarks. The same will happen in biology.

The middle-term problem rests with governments: how to set up a productive biotechnological community involving both basic biologists and industry, so that interested biologists can 'get their feet wet' and solutions be found to real practical problems. As with any technology, there will always be a need for an academic bioengineering community dealing with long-range issues of only partial interest to industry, between the industry-based biologist and his or her fundamentally inclined colleague; it is for government — and the universities — to set up this community and ensure its proper recruitment, training, and regeneration. In the UK, it is to be hoped that the Royal Society committee currently inquiring into biotechnology will make recommendations on this matter.

But strangely the long-term issue rests again with the biologists. The physicists have learned, rather late, that their technologies have had an immense impact on society. That impact began with a certain selection of priorities, mainly by industry, which led physicists to research in one area rather than another. (Thus, for example, solid state physics was vigorously pursued because of its significance to the electronics industry, whereas oceanography was relatively neglected — until the need to detect nuclear submarines or search for minerals.) The same forces are undoubtedly at work now in biotechnology — and this is precisely the moment when patterns of research will be formed which will ultimately shape lives. Communities and laboratories will be set up in various targeted disciplines, some fields will be encouraged and others neglected; expertise and training will develop along lines which will later be difficult to change. It is an interesting moment for biologists: they have great power in their hands. Do they let the entrepreneur guide them, willy nilly, to the fastest return? Or do they, if ever so slightly, change his priorities? □

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United States

David Dickson reports from the American Association for the Advancement of Science's annual conference in San Francisco.

New effort urged to control nuclear weapons spread

RECENT events in Iran and Afghanistan, and the domestic political response to these events in the US, have increased the need for scientists to support efforts to control the proliferation of nuclear weapons, a succession of speakers told a forum on ending the nuclear arms race.

Talking two days after President Carter had requested the US Senate to postpone its debate on the ratification of the Strategic Arms Limitation Treaty (SALT II), the speakers warned of the dangers of linking the nuclear weapons issue to broader political considerations of East-West relations.

"The present crises show that no security has been gained by massive nuclear arsenals. In fact the situation is only made more dangerous," said Professor Everett Mendelsohn of Harvard University. "I believe that scientists, who have been so especially involved in the design and construction of nuclear weapons and advanced missile systems, have an added responsibility to press for nuclear disarmament now. The scientific community has been too quiet for too long on the nuclear arms race."

Jeremy Stone, director of the Federation of American Scientists, emphasised that the importance of separating SALT from detente, as well as the whole logic of arms control, had never been so clear. "Supporters of SALT will have to, and should, separate their case for SALT from their case for detente. And SALT opponents will have to consider how far they want to go in giving up hopes for reducing the risks of nuclear war in order to ensure sharp US responses to Soviet adventures."

Reflecting the shifting political climate with respect to arms control, and military spending Dr Bernard Feld, editor-in-chief of the *Bulletin of the Atomic Scientists*, pointed out that the magazine had recently decided to move the clock on its front cover, an indicator of the world's proximity to nuclear destruction, from nine to seven minutes before midnight.

"This seemed an adequate gesture a few months ago, but after the recent events we should probably move it even closer, perhaps to five minutes to midnight," said Dr Feld. Reasons for the decision to shift the clock, which had previously remained unchanged since 1974, included the apparent breakdown in the SALT process and the spread of new nuclear weapon systems in Europe.

Professor Mendelsohn told the audience that a new *ad hoc* committee was being established to coordinate the lobbying efforts of scientists in pushing for nuclear weapons control, and urged that the AAAS council adopt a resolution recognising the importance of continued support for the SALT treaty. □

'Big brother' approach to space must go

THE US should support the efforts of the United Nations to forge an international consensus on the commercial uses of space, as this was the only way to guarantee long-term access to the markets being opened up by current research, according to Dr Jerry Grey, administrator of public policy for the American Institute of Aeronautics and Astronautics.

Referring in particular to a draft treaty of the uses of the moon approved by the UN General Assembly in New York last month, Dr Grey said that it was important for the US Senate to ratify this treaty, even though many scientists and industrialists had already expressed concern about some of the restrictions that it might place on the space activities of US corporations.

"If necessary, the US should express reservations in the process of ratifying the treaty. But we must hedge our bets as effectively as possible to ensure that our long-term interests are protected. We cannot afford to have the US barred from these activities as a result of charges of imperialism or of exploitation of the Third World," said Dr Grey.

He pointed out in particular that it was the US which had introduced the 'common heritage' language into discussion about the uses of space when the UN had first started discussing the issue in the early 1970s. Any other position than accepting the common heritage principle "would have seriously prejudiced US ability to conclude the cooperative agreements which are essential to US participation in potentially lucrative global markets," Dr Grey said. And awareness of this need among negotiators was one welcome sign that the US might be revamping its present "big brother" approach to global space applications.

In addition to the UN discussions, the recent successes of European space efforts emphasised how formerly sacrosanct US markets were being invaded. Unless policy-makers reversed their traditional reluctance to engage in cooperative activities, the US would almost certainly lose out on major forthcoming market opportunities. □

EPA wants more data on chemicals

UNLESS the chemical industry is prepared to present more substantial data on the health and environmental effects of new chemicals, the Environmental Protection Agency may have to take various measures — including if necessary temporarily preventing the manufacture of the chemicals in question — to ensure that adequate testing has been carried out, according to Dr Steven Jellinek, director of the EPA's office of toxic substances.

Dr Jellinek said that since the pre-manufacture notification requirement of the Toxic Substances Control Act had come into force six months ago, data on the testing of 51 new chemicals had been passed to EPA for approval. In examining these notices the agency had in particular found a tendency for companies to go for less expensive forms of tests, resulting in a relative lack of what EPA considered to be adequate safety information.

"In many cases, very little environmental or health data is included — for example none of the notifications received included data on chronic testing," said Dr Jellinek. "Unless the situation is corrected, our review of such notices is going to be based on inadequate data, and we will have to rely on our own studies, such as the use of short-term screening tests, which will mean that the information on which we base our decisions is very uncertain."

Dr Jellinek emphasised that it was not the intent of the FPA to stifle technological innovation through excessive regulation — indeed in many cases regulation had had the effect of stimulating money-saving improvements to production processes. However, in considering the objections of the chemical industry to the agency's activities, it had to be recognised that major breakthroughs in the industry were rare and that most innovation was of a relatively run-of-the-mill variety, which did not necessarily require the same reverential treatment.

However Dr Jellinek said that the aency was considering various ways in which it could "fine-tune" its regulatory efforts, including the possible exemption of certain little used chemical substances from the full requirements of legislative controls, and enabling generic pre-manufacturing notices to be granted on a range of closely-related products.

Earlier in the session, Dr William J McCarville, director of environmental affairs for the Monsanto Company, demanded the separation of scientific decisions from political decisions in the regulatory process — and suggested the creation of a high-level independent scientific panel to answer the scientific needs of all federal agencies that regulate chemical carcinogens. □

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United Kingdom

Ex-energy secretary criticises decision-making

FORMER UK Secretary of State for Energy, Mr Tony Benn, has broken the unwritten rules on public exposure of parliamentary procedures by giving an extended interview to Granada television (*World in Action* shown on 7 January) about key decisions taken during his 11 years as a senior Cabinet Minister. According to *World in Action* reporters, Whitehall officials have refused to respond to his criticisms on the grounds that he is breaking a long standing tradition that disputes should be kept behind closed doors.

Mr Benn cites five important issues on which, as the person in charge of Britain's nuclear programme, he was either not informed, misinformed or actively opposed by government bureaucrats.

The 1978 AGR-PWR decision: Benn favoured the British designed advanced gas cooled reactor (AGR) over the US designed pressurised water reactor (PWR) — of the type that failed at Harrisburg — on the grounds of safety. He told *World in Action* that he asked his civil servants to prepare a Cabinet paper in favour of the AGR. Instead, in conjunction with industrialists like Sir Arnold Weinstock of the General Electric Company who had obtained the UK license to build PWRs, they argued that the PWR had better export potential to countries like Iran. His department, he claims, then refused to help his political advisors prepare a brief in line with his views, and the Central Policy Review Staff (think tank) circulated a paper to other ministers directly opposing them. In this case Benn won the argument in favour of AGRs because he "was also making great efforts to see that other ministers knew what this battle was about."

The major Windscale leaks: In 1976, during discussions of expansion of the Windscale nuclear reprocessing facility, Benn heard about the major silo leak from the newspapers. In March last year, he responded to the 30,000 curie-300rem/hr sump leak by calling for a public inquiry in spite of what he describes as "intense political pressure" by the bureaucrats.

The UK-Namibian uranium deal: In 1970, officials in the Department of Energy approved contracts to let the UK nuclear industry obtain uranium from the Rossing mine in Namibia. Namibia was illegally occupied by South Africa and the Labour Government had insisted that it be informed before any contract was signed. The cabinet was not informed of the department's action by what Benn describes as a frequent bureaucratic ploy. Signed contracts are placed in "one or two or perhaps three red boxes a night' without drawing the ministers attention to their importance.

The 1968 theft of 200 tonnes of uranium from Euratom: The theft, from the ship Sheerbourg, bound for Genoa from Antwerp by presumably Israeli agents, was not reported to Benn when he was Energy Secretary on the grounds that Britain was not a member of Euratom.

The Russian Kyshtym nuclear disaster in the 1950s: This was not made public until 1977, although Benn claims that the information was known to the CIA who passed it on to the UK Atomic Energy Authority. The UKAEA did not tell Benn about the accident because, he claims, it feared that the accident "might throw doubt upon the safety of nuclear operations worldwide."

Benn feels that the only remedy to "the state within the state" is to expose the operation of government to public debate.

Joe Schwartz

NEWS IN BRIEF

Japan produces enriched uranium

THE Japanese government announced on 26 December that it had produced enriched uranium from its own factories. Previously, Japan has relied on the US for its supplies of enriched nuclear fuel. A factory in Ningytoge in the western part of the country has produced 300 kg of uranium with a 3.2% concentration of ^{235}U the fissionable isotope of uranium. (^{235}U has a natural abundance of 0.72%). By 1981 the country is expected to produce 50 tonnes of enriched fuel annually. The uranium will be used to fuel Japan's rapidly expanding nuclear programme which envisages the construction of an additional 15 power plants in the next five years, a doubling of present capacity. Enriched uranium at this level can also be used to construct fission bombs.

Evidence on laboratory animals invited

A UK House of Lords select committee which will consider revision of the Cruelty to Animals Act of 1876 has invited written evidence from interested individuals and organisations on current practices in the use of laboratory animals. The committee seeks answers to three questions:

- Is present law and administration for controlling the number and use of laboratory animals unsatisfactory?
- If present law needs admendment, what changes ought to be made?
- Does the proposed House of Lords Laboratory Animals Protection Bill meet the need for reform?

Evidence should be sent before 24 January 1980 to the Clerk, Select Committee on the Laboratory Animals Protection Bill, House of Lords, London SW1 0PW.

Neurochemistry society wins action

THE International Society of Neurochemistry has won its legal action against Pergamon Press to prevent it from continuing to publish a journal with the same name as the society's journal. The dispute arose when the society moved the *Journal of Neurochemistry* from Pergamon to Raven Press earlier this year. After completing a ten year contract with Pergamon, the society sought another publisher because of dissatisfaction with Pergamon's "long publication times" and the "inadequacy of its accounting procedures". Pergamon had insisted that it had the right to publish a journal of the same name and had sent a mailing to subscribers calling for continued subscription to the journal. It had also held back 50 manuscripts whose copyright is held by the society and had refused to release the subscription list. Pergamon now will hand over the subscription list and the unpublished manuscripts and will refrain from publishing any journal under the title "Journal of Neurochemistry". The society now will seek recompense for the 800 non-renewed subscriptions due to the dispute and for its costs in communicating with subscribers about the dispute.

Former Volvo director appointed head of ESA

THE Council of the European Space Agency appointed Mr Erik Quistgaard to the post of Director General at its 19 December meeting. Mr Quistgaard, aged 58 and a Danish national, graduated as a mechanical engineer (MSc) from the Technical University, Copenhagen. He spent three years with the Chrysler Corporation in the US and was General Manager and later director of Volvo in Sweden. After leaving Volvo he became Managing director of Odense-Lindo Stalskibsvaerft A/S, the Danish shipbuilding firm. Quistgaard will replace ESA's present director, Mr Roy Gibson, on 15 May 1980.

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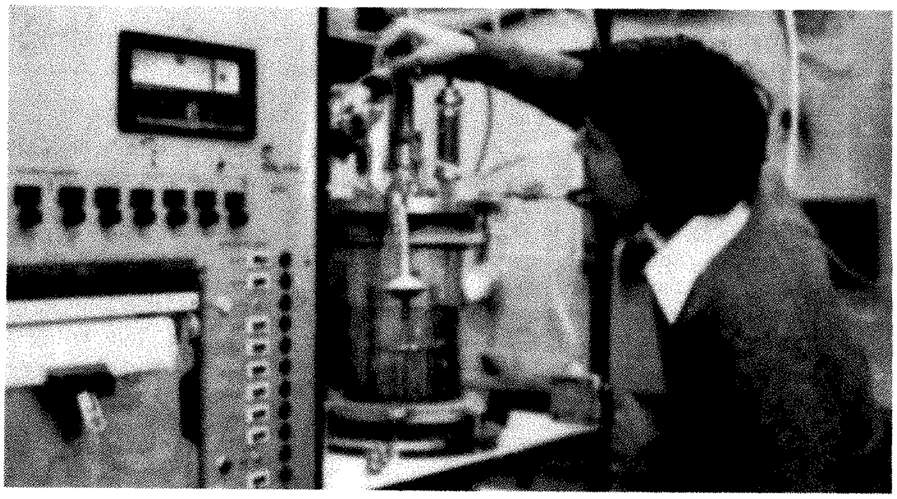
The biology business

In the first two articles of our special section, we look at the industrial potential of biotechnology and who is likely to exploit it first. **Professor Brian Hartley** of Imperial College, London is optimistic that it will be applied rapidly to many industries — he outlines its promise below. And opposite, **Robert Walgate** compares the efforts of different countries throughout the world



Tuning fermentation conditions before pilot plant trails

The bandwagon begins to roll

WHY should industry and governments think seriously of investing in biotechnology?

First is the tremendous rate of development of the technology itself. Five years ago, even optimists would have predicted that it would be a decade before genetic engineering could be commercially exploited, but this is about to be disproved. The earliest prospect is for new pharmaceuticals made cheaply by fermentation. Human hormones, enzymes or antibodies will replace animal products or create new therapies. Already bacterial fermentation processes for human somatostatin, growth hormone and insulin are patented and there is a world-wide chase for cheap interferon, potentially the wonder-drug of the 80s. Less dramatic, but equally important, is the prospect of new, cheap, safe and effective vaccines. By separating the gene for a viral antigen from the toxic or infective components, this harmless protein could be made cheaply by a microorganism. Such inherently safe products should also reduce expensive toxicity testing. Already progress is reported towards hepatitis B and influenza vaccines, and one can envisage that even measles and the common cold may succumb. Cheap animal vaccines will increase agricultural productivity: the prospects of such vaccines for foot and mouth disease or swine fever now merit consideration of programmes to eradicate these diseases.

Genetic engineering also offers new diagnostic tools to medicine. Enzyme or antibody diagnostic kits for the general practitioner are already a thriving business, and genetic engineering promises novel and cheaper products. Tiny amounts of the DNA or RNA of viruses and microorganisms can now be readily mapped, isolated, sequenced and replicated. Thus new subtypes of viruses can be recognised by their different DNA sequences and "probes" of DNA complementary to these will be invaluable in diagnosis and epidemiology.

The second major factor favouring biotechnology is the dramatic shift in terms of trade, and in public opinion, from fossil fuels to renewable energy resources. Environmental pressures have also forced up the costs of disposal of agricultural and industrial wastes: biotechnology can help by converting these into useful products. The simplest conversion is into microorganisms for animal feed (Single Cell Protein: SCP). For example, a British Petroleum process utilises n-alkanes — a side product of oil refining; a Shell process uses methane, also frequently wasted in oil recovery, and Imperial Chemical Industries grow organisms on methanol obtained from methane. Only the latter is currently judged economic, and then only by virtue of large-scale centralised production.

As petrochemicals become more expensive there are even brighter prospects for biotechnology in production of bulk chemicals. Japan has led the way with a profitable fermentation industry producing amino acids, nucleotides and organic acids, but this relies largely on conventional microbiology, genetics and fermentation feedstocks. It can certainly be challenged by new biotechnology applied to new sources of biomass. Indeed since many microbial products — proteins, polysaccharides, organic acids, for example — could be produced more cheaply than petrochemicals, should we not visualise new biopolymers, biosolvents, and biodetergents?

In this light, enzyme technology will surely take off. Genetic engineers already have ways to persuade microbes to superproduce enzymes of choice and even to secrete them into the medium. Alternatively the superproduced enzymes can be retained within the cell, so that the immobilised cell itself becomes a fixed catalyst. An early example is the ICI process for producing high fructose syrups with immobilised glucose isomerase. This has received the EEC seal of approval by earning a tariff because it competes effectively with sugar beet. Enzymes embedded in membranes can form enzyme electrodes for continuous monitoring of flow processes, and one can foresee membranes that are specifically permeable to a single compound.

Not least is the contribution that biotechnology may make to the world's energy problem. In hot, sticky countries such as Brazil, big schemes are already afoot for producing ethanol from sugar cane or cassava, as a fuel for cars. Moreover big improvements on the current conventional fermentation can be expected; for example, a rapid continuous fermentation by organisms living at high temperatures that would harness the heat of fermentation to distil the ethanol. Thus as the price of oil continues to soar, the Brazilian adventure seems to be far from a risk. Indeed, Western developed countries are beginning to examine their own resources of biomass for energy; industrial and agricultural wastes are an obvious target and, just around the corner are processes for converting cellulose into liquid or gaseous fuels. Then a really balanced alternative to our oil-based economy will have arrived. ☐

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Who's ahead, who's behind?

Between 1967 and 1971, when immobilised enzymes were emerging as the first wave of the new biobusiness, 30% of world biotechnological patents originated in one country: the UK. But of the patents delivered since 1977, 124 originated in Japan, 39 in the US, 9 in the USSR, 8 in West Germany, 7 in France, one in Denmark, and just one in the UK.

Armed with this rather depressing information for Europe, the European Commission requested two biologists — Dr Artur Rörsch of Leiden State University, The Netherlands, and Dr Daniel Thomas of the University of Compiegne, France, to produce reports* on the status and potential of genetic manipulations in applied biology, and of the production and exploitation of biological catalysts.

Meanwhile in France, Professor François Gros, Director of the Institut Pasteur, Professor François Jacob, Nobel laureate and also at the Institut Pasteur, and Professor Peirre Royer of the Delegation Generale pour la Recherche Scientifique et Technique were producing a report* on biotechnology for the President of the Republic, Giscard d'Estaing.

All these reports carry reviews of the state of the art in biotechnology around the world; together they provide a worldwide picture.

Japanese domination

Japan has taken an incontestable lead in biotechnology. The company Tanabe Seiyaku is credited with the first application of immobilised enzymes in 1969. It used aminoacylase carried on DEAE-Sephadex for the continuous production of L-amino acids from acyl-DL-amino acids, reducing overall operating costs compared with a free-enzyme, batch process by 40%.

Now five companies are in active production, in processes involving four different fixed enzymes — aminoacylase, aspartase, glucose isomerase, and penicillin acylase — and three fixed cells — *Escherichia coli*, using its aspartase metabolism, *Brevibacterium ammoniagenes* (fumarase) , and *Streptomyces* sp. (glucose isomerase). The Tanabe Seiyaku company has also developed processes involving fixed cells of *Pseudomonas putida* (L-arginine deiminase), *Achromobacter liquidum* (L-histidine ammonialyse), and *E. coli* (penicillin amidase). Many biotechnologists believe that the fixation of cells and cell organelles is the way forward for enzyme technology, and perhaps also for the use of genetically engineered microorganisms.

Japan's industrial interest has to some extent been stimulated by the numerous traditional fermented food industries, producing miso and shoyu and the drink sake through the *Aspergillus oryzae* amylases. The average consumption of miso is apparently 24 gm/person/day, and the producers have not been slow to attempt to capitalise on the new biology. The fermentation industry represents some 2-3% of Japan's GNP.

Outside industry, government has also taken a strong interest, particularly through the Office for Life Science Promotion headed by Professor A Wada. This office has set up a systematic programme for the research and development of enzyme technology, and has made a detailed analysis of its possible impacts on industry and society. "The association between a well-defined plan and the Japanese potential is unique in the world" writes Thomas in his report.*

The recently published French report* suggests that Japan has chosen to make an all-out effort on biotechnology because of the potentially high added value the technology can give to imported raw materials. In a country as dependent on imports as Japan this is an important consideration.

Furthermore Japan already enjoys "a very high reputation as one of the principal world producers of antibiotics" through fermentation, and the country intends to maintain this position through its research. "It is clear that in Japan there is no official or conceptual division between basic and applied research; there is a continuous exchange of skills and services between universities and industry".

However there is a feeling that Japan is not as advanced as some other countries in fundamental biology, so international competition particularly through genetically engineered organisms is not closed.

US catches up

America is the second most advanced country in this field, judging by patents and production. "The patent list shows that industrial interest in enzyme technology is significant, but so far the only large scale application is the production of fructose syrups by several companies" writes Thomas.*

But he forecasts an "explosion" in applications which should flow from the Research Applied to National Needs programme of the National Science Foundation. A strong interdisciplinary team at the MIT has been focusing on the total enzyme synthesis of gramicidin, a cyclic decapeptide antibiotic. This problem tackles head on the major obstacle to application of synthetic as opposed to degradative pathways: the cost-effective provision and regeneration of small cofactor molecules such as ATP and NADH.

The country that finds a practical solution to this problem, and patents it, will find itself at a major advantage compared with others; but there is some scepticism about whether extra-cellular systems will ever be successful.

Some eight cell species have been successfully immobilised in the laboratory in the US: *Streptomyces venezuelae* and *Bacillus* sp (for producing fructose), *Saccharomyces cerevisiae* (invert sugar), *E. coli* (fumaric acid), *Nocadia erythropolis* (Δ^4-cholesterone), *Corynebacterium simplex* (prednisolone), *Serratia marcescens* (2-keto gluconic acid), and *Corynebacterium lilium* (glutamic acid).

Moreover the US is well-advanced in applied recombinant DNA techniques — the production of somatostatin for research purposes being promised shortly, with insulin also on the way; and it has a very strong base in fundamental biology. Research and development organisations based on venture capital are very active (see *Nature* **278**, 494; 1979), and at least one (Genentech) is active in recruiting scientists outside the US, particularly in Europe.

Soviet secrecy

Soviet commitment to biotechnology, indeed to all aspects of molecular biology, began officially with the decree of 21 May, 1974, which set Soviet science the task of "accelerating the development of molecular biology and molecular genetics" and the "wide use of their achievements in agriculture, medicine and industry". Considerable human and financial resources were committed to closing the

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**"Genetic manipulations in applied biology" by A Rörsch, catalogue number CD-NI-78-001-EN-C; "Production of biological catalysts, stabilization and exploitation" by D Thomas, CD-NI-78—002-EN-C, available from offices for official publications of the European Communities; and "Sciences de la vie et Societe" by François Gros, François Jacob, and Pierre Royer, available from La Documentation francaise.*

gap left by Lysenkoism.

Partly as a result of Lysenkoism, Soviet "applied" genetics has a strong commitment to radiation-produced mutations. However, several groups throughout the USSR are known to be working on applied genetic engineering, and there have been several hints that the industrial breakthrough point for "certain medical products" made in this way will be reached "in the nearest future".

Most socialist countries, however, are somewhat secretive as to detail. One exception is Hungary, with its international biology centre at Szeged (built largely by UNDP funds). Work at Szeged, however, is still largely at the stage of establishing techniques and principles. One team, researching on nitrogen fixation, recently started work on establishing a genetic map for *Rhizobium* and studying the genetic structure of chemically produced mutants.

In Poland, however, the Institute of Biochemistry and Biophysics of the Academy of Sciences would put the breakthrough point for nitrogen fixation by "agricultural plants" at the order of 20 years ahead. According to interviews which specialists from the institute recently gave the prestigious weekly *Polityka,* they would put on the same time-scale the modification of cereal genes so that the grain proteins would provide the whole range of aminoacids needed for human nutrition. In the "near future", on the other hand, they expect the commercial production of hormones (unspecified), vaccines and special microorganisms designed to clean up pollution. **Vera Rich**

Germany leads in Europe

A major initiative in the European Economic community is discussed opposite — but, in outline, Europe starts well behind Japan and the US in the application of modern biology. Within Europe, West Germany is generally reckoned to be in the lead: government spending on the basic problems of biotechnology is about ten times that in France or the UK, for example. Altogether, between 1972-78, the federal government invested £35 million in biotechnological R&D.

One of the highlights of the German effort is the Gesellschaft für Biotechnologische für Forschung mbH at Braunschweig near Hannover, with an annual budget in 1977 of some £5 million. It was created in the early days of biotechnology — the mid sixties — by the Volkswagen foundation, but was later taken over by government. It has a staff of some 80 researchers and support staff, but has been criticised by some for being insufficiently productive. A new director, Professor Kieslich, has recently been appointed.

The science ministry (Bundesministerium für Forschung und Technology) also plays an active part in funding and planning biotechnological research and development in university and industry; its programme is the envy of biotechnologists in other European countries. So far Germany's greatest efforts lie in its government based research programme.

Italy has a fairly advanced programme, particularly through the activities of the firm SNAM-Progetti, which operates a large laboratory devoted to biotechnology at Monterotondo, near Rome; and Sweden can boast ground-breaking work at the Universities of Lund and Upsalla. In Switzerland Nestlé "is devoting a big effort" (Thomas). (UK and French programmes discussed opposite). ☐

The origins of biotechnology

THE most ancient biotechnological art is fermentation — in the raising of dough, the brewing of alcohol, and the production of tea, coffee, and cocoa (each of which requires a fermentation step). But the new wave of biotechnology rests on two main techniques: genetic manipulation and enzyme (or cell and cell organelle) immobilisation.

Genetic manipulation is used by the biotechnologist to enhance the natural genetic repertoire of a microorganism. The aim is usually to donate to the microorganism a gene for an enzyme or a hormone in such a way that the product of that gene is thenceforth synethesised by the microorganism. The successful development of genetic manipulation has rested upon the discovery of new enzymes and the improvement of old techniques by molecular biologists and microbiologists.

Crucial to the success has been the development of vectors for carrying foreign genes into microorganisms. Also of great importance has been the acceleration of DNA sequencing technology.

The real cornerstone of genetic manipulation, however, has been the discovery of enzymes that cut DNA at specific sites (restriction enzymes), seal the cuts up (ligase) and copy DNA from messenger RNA (reverse transcriptase). The first two of these are needed for the insertion of foreign genes into the DNA of the vector. Reverse transcriptase has become particularly important in the last two years since the revelation that the genes of higher organisms are 'split' (the coding DNA being interrupted by non-coding segments). In sharp contrast, bacteria do not have split genes and cannot decode split genes that are transferred to them. Therefore it is necessary to transfer an unsplit equivalent of the gene, which is precisely what reverse transcriptase produces from messenger RNA. Alternatively, due to recent advances in the technique of DNA synthesis, it is also possible to make purely synthetic genes at least for small peptide hormones.

The use of enzymes is an older technique, applied in a number of industries from baking to detergents. But purified enzymes are soluble molecules and it is therefore not easy to separate them from the product of their labour. Furthermore it is difficult to recycle the enzyme. These difficulties have led to the development, in the late 1960s, of immobilised enzymes. The enzyme is bound chemically to a solid matrix or entrapped in a small-pore gel.

Immobilised enzymes have been successfully applied, for example, to the production of semi-synthetic penicillins, to the large-scale production of fructose from maize, and to a simple assay for blood cholesterol.

Immobilised cells or organelles have potential advantages when the stabilisation of an enzyme is difficult, or when a coenzyme or sequence of enzymes is required for synthesis.

Biotechnology may be dominated by microbial and enzyme technology, but it is certainly not synonymous with them. Both animal and plant cells have their place in the armoury of the catholic biotechnologist. Successful exploitation of animal cells has been achieved, for example, by Wellcome Research Laboratories in the production of interferon from virus-stimulated lymphoblastiod cells.

A completely distinct exploitation of animal cells lies behind the commercial production of high grade antibodies for research, diagnostic and clinical use (for example for tissue typing prior to transplantation). The key to that is the hybridoma cell, an 'invention' of five years ago. Natural antibody-secreting cells neither survive long enough in culture nor produce pure enough antibodies to be a commercial source of antibody. But a hybridoma cell, the result of fusing an antibody-producing cell with a tumour cell, grows and divides continually. And all the cells from the original hybridoma secrete the same, pure antibody.

Work is also going ahead in many countries on the problem of nitrogen fixation — attempting to extend the range of associations of the nodule-forming, nitrogen-fixing, bacterium *Rhizobium* to plants other than the *Leguminosae* (particularly cereals), or to transfer the nitrogen fixing genes to plants — a distant prospect, given the extreme oxygen sensitivity of the pathway. There is also the distant prospect, through genetic manipulation, of a new source of controlled genetic variability in plants to produce new high yielding or resistant varieties.

Peter Newmark

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Brussels asked to spend £16m on biotechnology

Today, the EEC's thirteen Commissioners are being presented with a proposal for a five year (1981-85) community programme for research and development in 'biomolecular engineering'. Here, **Robert Walgate** outlines the proposal and the rationale behind it. Over the page, he summarises a report on biotechnical safety prepared for the Commission.

THE proposal being put before the Commission has been originated by a small team of officials in the research directorate of the Commission; the officials are themselves active in biological research, but their proposal is based on a wide range of advice from experts, government officials, and industry received over the last two or three years. It will only become official if first, it is approved by the Commission and second, it is accepted by member governments through the Council of Ministers. If all went smoothly, this process could be complete by the end of April.

The programme would be an 'indirect action', whereby the Community budget would contribute half the costs of research projects, and contracting parties the other half. The proposed Community contribution is 23.5 million European Units of Account (about £16 million) divided over nine countries and five years — crudely some £300,000 per year per country, although the allocation will be strictly according to the quality and suitability of individual applications. The amount is small compared to Germany's national effort, but would in the UK for example, match the Science Research Council's present expenditure — so the proposal is far from negligible. European industry has recommended its speedy implementation.

There would be six projects in the programme. They are designed in part to tackle basic technical obstacles to the development of biotechnology, and in part to suit particular European problems. The programme is outlined below.

French views

Professor Daniel Thomas, enzyme technologist at the new University of Compiègne, near Paris, is naturally in favour of an EEC effort: he wrote the report which forms the basis of the first and second projects. He feels one of the main advantages of an EEC action will be that it promotes cooperation. 'In Europe we have better contact with the US than we do with each other" he says. Cooperation might be difficult on development questions, but on basic research a coordinated programme would be "very helpful".

"There are some 4000 PhD biotechnologists in Japan; in France we have only about 200. We need a critical mass in Europe to compete with Japan and the US."

Fundamental biology is of a very high quality in Europe, Thomas believes, and though industry is at a low level, it is good enough to succeed "if there is a big effort". But Europe cannot hope to "reproduce Japan". The first ten companies in biotechnology in Japan have grown out of the food industry, which took an early enlightened attitude to the new biology.

In America, dedicated contract research organisations such as Biogen, Cetus, and Genentech were thought to be the answer "but it would be better in Europe to arrange loose links between academic institutions and industry". This is one of the objectives of the proposed EEC programme.

A big leap forward in biotechnology requires "both high level work and classical fermentation chemistry" says Thomas. The effective application of genetic manipulation is, he believes, a long term goal; "in the short and middle term we must look at enzymes and fermentation". The fundamental bottlenecks in enzyme technology could be solved — scientifically at least — within five years, he believes.

France has been slow to recognise the importance of biotechnology. "Before the report to the President (see footnote p123) the very words 'biotechnology' and 'enzyme technology' did not exist in

The proposed EEC programme

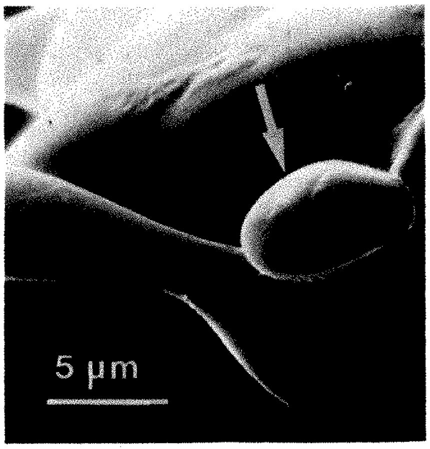
In bare outline, the programme consists of:

First project: enzyme technology, involving

- reactor kinetics and geometries for multi-enzyme reactions
- immobilisation of multi-enzymes and co-factor regeneration
- stabilisation of lipid-requiring enzymes and development of reactors for reactions at a hydro-organic interface
- stabilisation of sub-cellular organelles such as mitochondria, chloroplasts and peroxisomes and of membrane-bound enzymatic complexes in the form of microsomes, thylakoids, and inner mitochondrial and plasma membranes; and of intact eukaryotic plant and yeast cells.

Second project: development of methods for industrial and human detoxification, involving

- bio-compatible insoluble matrices
- targeted carriers, with specific tropisms, for administering medical enzyme products



Immobilised lettuce chloroplast: the EEC's energy programme is already supporting research on the production of hydrogen from immobilised chloroplasts associated to bacteria hydrogenase

- enzymatic detoxification of poisons.

Third project: transfer of genes to *E. coli*, the yeast *Saccharomyces cerevisiae*, and 'other suitable organisms', involving:

- overcoming expression barriers
- construction of 'synthetic genes'
- development of mutational tools to produce, for example, harmless vaccines or stabilised enzymes
- modification of enzymes to inhibit their degradation in a foreign environment
- development of 'libraries' of expressed genetic information and rapid screening techniques for their identification

Fourth project: development of cloning vehicles in organisms which are likely to be of greater importance to industry than *E. coli*, such as the bacteria *Actinomycetes*, *Pseudomonas*, and *Bacilli*, the fungi *Penicillium*, and algae, using plasmids, viruses, and mitochondrial DNA; and in plants using potential vehicles such as *Agrobacterium* plasmids, plant DNA viruses chloroplasts and mitochondria.

Fifth project: stability, regulation, and expression of transferred genes, including the regeneration *in vitro* of plant cells

Sixth project: improved methods for detecting contamination in fermented strains.

In each of the six projects, some three to 12 research groups would participate; each group would consist of, on average, three researchers, two technicians, one laboratory helper, and 'secretarial assistance'.

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France" says Thomas. Funds for his work at Compiègne came in small amounts from a dozen different sources. But now, after saturation coverage for two weeks on television and the newspapers "everyone was trying to do it".

But there are dangers in this sudden enthusiasm, he feels: biotechnology is not the panacea the media have taken it to be.

English disease

Looking over the water to England, Thomas is "very surprised" that the University College, London, group of Malcolm Lilly and Peter Dunhill " — the first in the field — get so little money".

Lilly, it should be said, does not complain at the size of his share of the small British cake in biotechnology, but is unhappy with the size of his group: "two more lectureships and an administrator would change the picture overnight" he told *Nature*.

Lilly's group is unique in the UK in combining chemical engineers and biologists — essential for good biotechnical work, Lilly believes. Biologists must feed the engineers with science, and the engineers feed the biologists with problems; and "ideas are transferred from person to person". Technology transfer is, Lilly feels, "bloody hard work!".

He spends about one day a week talking with industrialists: "one desperately wants good industrial/academic liaison".

There are two categories of biotechnologist in England: the recognised specialists, and people in associated fields who are attempting to find application for their work. "These people need guidance" says Lilly.

There are very many plausible applications, "but we have learned to be very selective in what we offer industry". Much work needs to be done between idea and application, and an ill-thought out idea will ultimately fail.

The number and age of biotechnologists is a central problem in the UK, he believes. "Most specialists are in their forties, and isolated in biochemical or engineering departments" where they have risked becoming black sheep. Very few departments have appointed younger people to follow them through. "The reason we've done better than others in the UK is that there were always two of us. We could talk to each other."

Lilly's group now consists of three full-time researchers, plus 20 technical staff and research students. "If I had two vacant lectureships I could fill them tomorrow" he says.

The potential economic productivity of such an investment is high, he believes. In the early days of enzyme technology in the UK some £500,000 was spent on research grants. The result was industrial immobilised enzyme processes, of which the biggest was fructose production, then the deacylation of benzyl penicillin.

For the future the main problem is handling co-factors in biosynthetic pathways. But here one should not distinguish enzyme and cell immobilisation: for example with glucose isomerase for fructose production some firms use cells, others the isolated enzyme. Where co-factors are required, whole cells have the edge. Fermentation also will remain important, particularly for methane production and waste treatment.

The key to industrial success, Lilly believes, will be to look for products of high added value, insulated from volatile market forces. For instance, the fermentation of sugar to produce industrial alcohol "is at the whim of the weather", which affects sugar prices; and single cell protein is similarly affected by the price of conventionally grown soya protein with which it competes as fodder.

Professor Lilly may take heart from the signs that the UK is about to make a second effort in biotechnology: a Royal Society committee on biotechnology is about to report, there are moves afoot to set up a British version of the American Biogen company, and the National Research Development Corporation is reportedly poised to make an increased investment — more in proportion to its revenue from biological patents, which greatly exceed its revenue from physical technologies.

And at the Science Research Council, which currently spends some £300,000 a year on biotechnology, a joint panel is shortly to be set up involving industrialists and academics; the panel will recommend by the Autumn a coordinated programme in biotechnology. According to Dr Geoffrey Potter, chief administrator of the SRC's Engineering Board, one of the main objectives will be to train new biotechnologists, or to help existing biologists shift towards technology. □

How safe will biobusiness be?

"THE cow," write two biologists invited by the European Commission to produce a report on the hazards of biotechnology, "is a live, mobile, self-reproducing and edible fermenter." To digest grass and produce milk it employs a complex and varying mixed culture of anaerobic microorganisms in its rumen; some of these have not even been characterised.

"The cow can become infected with pathogens, including foot and mouth disease virus, *Brucella abortus,* and *Mycobacterium tuberculosis*, which can damage or destroy the fermenter and infect the product. In the past it has been a major source of human brucellosis and tuberculosis.

"A singular disadvantage of this fermenter system is that it can only be scaled up by increasing the number of units; moreover its productivity, in terms of protein production, is only 0.0025 compared with that of an equal weight of microorganisms growing in an industrial fermenter."

Conjectural hazards of supposedly 'unnatural' techniques are not of happy memory for molecular biologists who alerted the world to what are now widely accepted as the largely imaginary risks of research using recombinant DNA. But painful though it may be, the questions must be re-thought if industries based on these and other new biological techniques are to be set up.

The scale of industrial processes, using 1000 to 30,000 litre fermenters, is vastly greater, and process control is more difficult. Also there is an economic disincentive to using severely disabled strains, and little attention has been paid to the dangers of the use of plant pathogens.

These and other issues have been raised in the report quoted above by Drs K. Sargeant and C.G.T. Evans of the Microbiological Research Establishment, Porton, UK. They conclude that:

- The special hazards of biobusiness rest only in the potential of some microorganisms to infect higher species and cause disease.
- "With the exception of a few that affect plants and insects, it is very unlikely that dangerous pathogens will be used on a large industrial scale."
- Research is needed on the long-range airborne transmission of, especially, plant pathogens.
- "For diagnosis, research and vaccine production some highly dangerous human and animal pathogens must continue to be grown on a small scale. A list of such organisms should be drawn up, and their handling, except under licence, should be forbidden. . ."
- "The chemistry of a proved and licensed fermentation process can alter in response to genotypic and phenotypic changes in the culture. These can be difficult to detect, especially in long-term continuous processes."
- Hence, research is needed on the rapid detection of small numbers of contaminant microorganisms within large populations; and on the mechanisms of, and potential for, phenotypic change.

On regulation of the industry in Europe, Sargeant and Evans argue that biotechnology will be "too diverse to be encompassed by a single set of regulations". Rather, they recommend that "regulations should be worked out by associations of manufacturers of product types in conjunction with governments, and harmonised within the EEC. This is already happening for food enzymes and single cell protein".

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In their review of hazards, Sargeant and Evans keep the problem firmly in perspective. ''Pathogenic microorganisms are a normal feature of our environment, held in check by Public Health measures and an informed food technology.'' The dangers of large-scale biotechnology must be seen against this background.

The main new features are scale of production, the large numbers of workers exposed to the organisms and their products, and the possibility of inadvertent release affecting the environment.

'Avirulent strains' may not be what they seem. ''Avirulence is a matter of degree, related to dose, portal of entry, and resistance of the host. Although so far unknown, the enhancement of virulence by phage transduction is a possibility. . . that should not be entirely ignored.''

The production of diphtheria toxin, the authors point out, is associated with the presence within *Corynebacterium diphtheriae* of a bacteriophage.

Further, the contents of the fermenter may not be what they were thought to be. They may become contaminated with foreign organisms (unless the fermenters are well closed), or there may be phenotypic variation.

One of the main ways the latter could occur is inadvertent change of the growth limiting nutrient, perhaps by failure of the supply line of a medium component. ''Cell walls of rapidly growing phosphate-limited *Klebsiella aerogenes* NCTC 418 are only slightly toxic to mice whereas similar, but carbon limited cell walls are highly toxic.''

The change from batch to continuous culture can also change the character of an organism: batch grown cells of the live vaccine strain of *Francisella turlarensis* are much more toxic to mice than the continuous grown cells, although the protection is equal.

Change of temperature can also be important. At 15°C and 45°C the aflatoxins are produced by *Aspergillus flavus* in only trace amounts on groundnuts; at 35°C, under otherwise identical conditions, 40mg/kg are produced. And change of *p*H through failure of the control system — ''a not unknown occurrence'' — can also cause major phenotypic changes. Change of trace elements in the feedstocks may also stimulate or inhibit the production of particular metabolites.

Sargeant and Evans illustrate the danger with ''an entirely hypothetical, but easily credible, example''.

''Until 1960, *A. flavus* was regarded as a harmless mould closely related to the *A. oryzae* strains used in Japan for making miso, shoyu and sake. It would not have been unreasonable to think that a pure culture of *A. flavus* could be eaten with impunity.

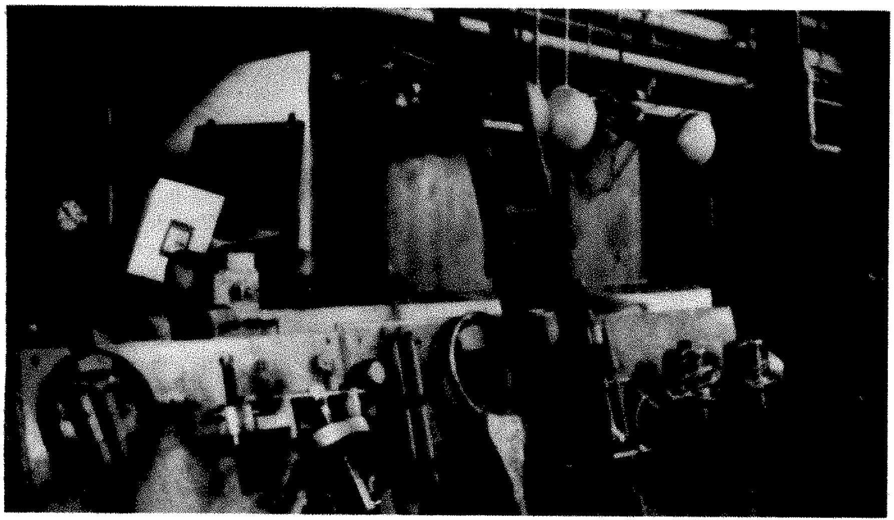
''In 1960, major outbreaks of a 'new' disease among turkey poults and ducklings — turkey X disease — were encountered in the UK. The cause was traced to a new group of toxins, now known as the aflatoxins, which were generated on contaminated groundnuts by some strains of *A. flavus*.

''It has since been shown that aflatoxins can cause serious disease in many animals, including man; epidemiological studies suggest that they are a major cause of human liver cancer in some parts of the world.

''*A. oryzae* was being developed in 1960 as a source of amylases for bread making, but it could have seemed reasonable to develop *A. flavus* instead. Alternatively a potentially toxigenic strain of *A. oryzae* could have been used, and, by chance, growth conditions that did not allow aflatoxin production could have been chosen.

''After careful testing, the product could have been declared safe. Subsequently, a small inadvertent change in the growth conditions could have led to the production of very heat stable aflatoxins and to their inclusion in our daily bread.''

However, those primarily at risk are the workers, subjected to ''the dense aerosols of microorganisms which, in the absence of suitable precautions, will be released into the factory atmosphere during recovery of the products of fermentation by centrifugation, spray, or flash drying, packaging and handling of the product, and release of the culture supernatant.''



Hard-headed about safety: inside a biotechnical laboratory in London

Outside the factory residents are no more likely to be at risk than those near a mechanically aerated sewage works ''with its potential population of human pathogens''.

The products of enzyme-based factories — such as the high fructose syrups used in soft drinks — may be contaminated with enzyme, and the effect of long-term low-level exposure is unknown. But the non-biological contaminants of soft drinks — such as sugar and phosphoric acid — may themselves have greater deleterious effects.

Man's environment could be affected by the discharge of nutrients, increasing microbial, algal and plant growth, but this problem is not exclusive to biotechnology.

The discharge of live organisms might have ecological effects, but since the strains will be unnaturally specialised their release would be ''tantamount to abandoning an over-bred and pampered European lap-dog to its fate in Siberia. We have been authoritatively informed that when, some years ago, the contents of a very large antibiotic fermenter were inadvertently discharged into the sea, no trace of the organism could be detected the next day, despite a very thorough, widespread, and anxious search.''

More dangerous might be the continuous low-level discharge of antibiotic, creating ideal conditions for the selection of resistant organisms. If contained on a plasmid that information could be transmitted throughout the microbial world. However, the widespread use of antibiotics in animal feedstuffs is a greater danger, the authors suggest.

The use of plant pathogens in increasing: ''the 400-litre production scale of the anti-leukaemia enzyme asparaginase from *Erwinia chrysanthemi* — pathogenic for chrysanthemums — has been completely overshadowed by that of xanthan gum from *Xanthomonas campestris* — pathogenic for brassicas.''

Most serious is the potential airborne dissemination of such organisms to countries in which they are not indigenous; ''and their aerosol survival and infectivity have been attributed to the production of the very gum for which they are grown''.

Finally there is the danger of the establishment of new genotypes during continuous cultivation, where organisms are maintained through many thousands of generations.

''If a contaminant arose which was able to maintain itself...then no guarantee concerning either the safety of the process or the culture could be given.

''The situation is in this respect fundamentally different from farming with plants and animals, where much more precise control of the genotype is possible — because one generation is cultivated at a time and alterations in the genotype are...much more obvious.''

Robert Walgate

Patenting living organisms — how to beat the bug-rustlers

Attempts to patent 'live' biological processes have raised wide debate about the legal and philosophical implications. But patent protection may prove less attractive than a strategy of closely-guarded research and swift commercialisation. **David Dickson** reports from Washington

FIVE days before Christmas, the Upjohn Company dropped a firecracker from one of the most keenly-awaited decisions facing the Supreme Court — whether patents can be granted for living organisms.

Officials of the pharmaceutical company say they have withdrawn a patent application for a strain of bacteria capable of producing the antibiotic lyncomycin, first filed in 1975, to focus the court's attention on a second application involved in the same decision, filed by scientists with the General Electric Company.

Unlike the Upjohn bacterium, which is essentially the refinement of a strain discovered in nature, the GEC case involves a strain of bacteria of the *Pseudomonas* genus genetically altered to maximise its effectiveness in degrading oil spills — a manipulation which, although it did not make use of the now-familiar gene-splicing techniques, is still considered "genetic engineering".

Yet the Upjohn patent application had already been approved by the Court of Customs and Patent Appeals, overturning an earlier rejection by the Patent and Trademark Office. The company's surprise withdrawal is therefore a further indication of the extent to which the recent rapid marriage between modern biology and industrial production has caught traditional legal machinery unawares.

'The line between life and non-life is so fine as to baffle even the experts'

Furthermore, while lawyers and philosophers debate the subtleties raised by the private ownership of living matter, others claim that patent protection may be only marginally relevant. Greater profits can be found in a combination of closely-guarded research and rapid commercialisation of the results, closer to the strategy of advanced electronics firms than the conventional ways of doing business in the chemical or pharmaceutical industry.

Turning scientific knowledge into a commodity has produced relatively few problems of definition in the physical sciences. Biology has proved less amenable. The Appeals Court, in granting the two patent applications, found little difficulty in classifying microorganisms as "industrial tools", or in concluding that "life is largely chemistry" — but critics see it as the thin end of a wedge that opens the door to private ownership and control of the evolutionary process itself.

Congress has already faced this dilemma. In the 1920s, when plant breeders began pressing for patent protection for new strains of seeds, it decided that plants were not covered, and in 1930 it passed the Plant Protection Act broadening the definition of patentable matter to include certain varieties of plants.

The argument of those now seeking to patent microorganisms is that, as previously the case with plant biochemistry, modern biology has so blurred the distinction between animate and inanimate matter as to make the concept of life — in the strict legal sense — almost irrelevant.

"Surely where the line between life and non-life is so fine as to baffle even the experts in the art and at times cannot be drawn with conviction, compositions of matter or manufactures near the periphery cannot conveniently be deemed patentable or unpatentable on so ephemeral a ground," according to an *Amicus Curiae* (friend of the court) brief filed by the University of California at Berkeley.

According to such arguments, there is nothing special about life that makes it inherently unpatentable. "We do not see any sound reason for making the distinction between the living and the dead," wrote Judge Rich in giving the Appeals Court's reasons for approving the patent application.

According to the court, it is precisely because organisms are alive that they can be useful. "We see no sound reason to refuse patent protection to the microorganisms themselves, or to pure microorganism cultures — the tools used by chemical manufacturers in the same way as they use chemical elements, compounds and compositions — when they are new and unobvious."

The Solicitor General, in appealing the decision to the Supreme Court, has sidestepped the philosophical issues to argue that, whether or not living forms should be patentable, they are not covered by present legislation; that Congress's previous introduction of new laws covering plants was a recognition of this fact; and that given the wide social and ethical implications of the current cases, the issues should be resolved by Congress rather than the courts.

If the Supreme Court decides to pass the buck back to Congress — and this would be consistent with other recent rulings — the stage will be set for a further round of public debate on the social consequences of genetic engineering. And in this case, the focus will not be on their potential hazards, but who is entitled to benefit financially from their use.

Opposing the argument that the creation of substances by live processes should be treated no differently from ordinary mechanical production processes, critics say that sufficient protection for useful techniques can still be obtained without necessarily giving away title to complete organisms.

Dr Burke Zimmerman, previously a congressional staff member closely involved with attempts to legislate recombinant DNA research and now with the National Institutes of Health, believes that a law permitting the patenting of living organisms would lead to "irresoluble logical inconsistencies". "To patent a living organism that is to be used only in a very specific way is *not* equivalent to patenting the particular use one may wish to put it to," says Dr Zimmerman. "Thus the classical justification for patenting an invention or even a composition of matter breaks down when applied to a living organism."

Others go further to argue the moral case as part of a general critique of the undesirability of genetic engineering. It is strongly put by the People's Business Commission, a Washington-based research organisation. "To justify patenting living organisms, those who seek such patents must argue that life has no 'vital' or sacred property; that all of life's properties can ultimately be reduced to the 'physico-chemical,'" says the PBC in a brief filed in support of the Justice Department's appeal to the Supreme Court. Furthermore, says the PBC, any such decision could set a dangerous precedent. "If patents are granted on

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microorganisms there is no scientific or legally viable definition of 'life' that will preclude extending patents to higher forms of life," the group argues.

Yet whether the issue is settled by the Supreme Court or by Congress, many now argue that the patent system may be incapable of providing adequate protection. Although patents provide 17 years protected use, they require in return full publication of the procedures used as a form of public education. Many fear su disclosure could provide important clu potential competitors who, w directly infringing patent righ n quickly come up with riv the

Others argue that althoug ch. may be granted by an entary) prove difficult to de ow rely general ubiquity of in relative marketing

As an alterned a saleable strategy, mas spiritedly of primarily o "bug-rustling"; secrecy lity, rather than once ave become the keys

fors to vary across the those who feel that the e most important thing we ers who feel it is almost ant", says Nelson Schneider of investment analysts E.F. Hutton, a close observer of biotechnology developments. "The problems are more in the area of fears about what someone else is going to do, rather than seeing anyone else do it. I am aware of no-one who, if they made a really great breakthrough in the field, would immediately file a patent application — they would prefer to sit on it."

'The classical justification breaks down when applied to a living organism'

That market dominance can be as effective as patent protection has already been illustrated by the pharmaceutical company Eli Lilly. Last year Lilly, which currently has about 85% of the domestic insulin market, agreed with the Federal Trade Commission to make both supplies of animal pancreas and the appropriate refining technology available to anyone interested — yet such is the company's prominent position in the market, and the economies of scale it is therefore able to effect, that other companies have been reluctant to take up the challenge.

Eli Lilly is now engaged with Genentech in developing the artificial production of human insulin, with hopes that it could be on the market by the end of the year. And this speed is dictated partly by the need to

'. . . a philosophical reductionism already responsible for the destruction of natural ecosystems and alienation of humanity from nature'

tial competitors who may head off working o synthetic insulin also ion.

preary emphasis on marketing may be more necessary to maximise returns another new biotechnology which is xpanding raidly, the production of monoclonal antibodies with highly specific characteristics from hybrid cell lines. By fusing a mouse spleen cell and a rapidly-reproducing tumour cell, and selecting out those resultant cells producing antibodies with particular desired characteristics, Dr Cesar Milstein and Dr George Kohler at Cambridge, England, have opened the way to a reliable method for the general production of antibodies.

But even more than with genetically-manipulated microorganisms, the information contained in a patent application on a hybrid cell-line describing its usefulness may provide an idea of how the same result could be achieved by a slighly different route. "Even if it does turn out to be possible to patent cell lines, we are not sure that it would be worthwhile," says Dr Howard Birndorf, vice-president of Hybritech, a west coast firm set up with the same source of venture capital as Cetus and Genentech. It is already marketing monoclonal antibodies to the hepatitis B surface antigen, and promises many more. "Rather than going for patent protection, we have chosen to develop techniques and processes in-house, staying more under trade-secret type protection," says Dr Birndorf.

Once a technique is perfected, the strategy is to market the product as quickly and as widely as possible, attempting to build a quick lead over potential rivals. "In immunodiagnostics the person who is first traditionally gets the largest share of the market," says Dr Birndorf. And he compares the hybridoma field to the semiconductor industry, where it is the company that can raise the number of useable silicon chips from two to 30 in a hundred that wins out.

But while secretive research and aggressive marketing may suit the style of the small, venture-capital-based firms, they fit less easily into the traditional *modus operandi* of biological research.

Many biologists, caught up for the first time in what may turn out to be highly lucrative research, but uncertain of procedures and frightened of giving anything away unwittingly, have become confused about what to do. Others are accused of violating the spirit of the research community.

"Scientific meetings in this field used to be free and open," says one observer. "Now everyone sits on their hands in a corner, not talking to anyone else. There is a fear of what you may giving away — and how long it may take before you know what you have done."

Mr Neils Reimers, head of the Technology Licensing Office at Stanford University, claims this is as much the fault of the rush to publication as of the demands of the patent system. But he accepts that arranging the commercial exploitation of biological advances in ways mutally beneficial to the public at large, private corporations and the university scientists who carried out the original work had raised problems.

Even with the recombinant DNA techniques, once a patent has been granted, it may prove difficult to enforce. Stanford, for example, has already applied for a share of the patent rights to the original gene-splicing techniques developed by Dr Stanley Cohen and Dr Herbert Boyer. And if this is granted, various companies have suggested that they may challenge the patent if they feel the university is demanding too high a licence fee.

Such difficulties in enforcing patent claims mean that, despite the potentially lucrative discoveries now emerging from biology laboratories, universities may not find them the gold-mine that some have speculated. But there are already moves in Washington to assist universities if they do come up with a winner.

In a package of recommendations issued in November, and designed to stimulate industrial innovation, President Carter proposed among other things a revision of the patent law making it easier for universities and small businesses to retain the patent rights on discoveries resulting from federally-sponsored research.

This will be done through a system of what are known as Institutional Patent Agreements. To qualify for an IPA, a university must demonstrate an ability to transfer research results to the private sector, and must agree to use any income for research or teaching. In return it will be allowed to arrange the licensing of the patented results with a minimum of government interference.

Many current complaints about the patent system are addressed in a bill passed by the Senate last autumn, and sponsored by senators Birch Bayh and Robert Dole. If passed by the House, this will formalise a university's right to profit from the discoveries of its scientists in a manner simila to the Administration's proposals.

Others, however, are worried that t corporatisation of university research, a stretches down to the workings of the c may reinforce a philosophical reductionism already responsible, it is claimed, for the destruction of natural ecosystems and the increasing alienation of humanity from nature. ☐

The ties that bind or benefit

As industrial corporations become more involved in developing new biological techniques, where does this leave the scientist? How will university biology departments maintain their integrity and autonomy? How will individual scientists react t[illegible] and corporate demands? These and other questions were exp[illegible] when *Nature* brought together **Sheldon Krimsky**, Acting Direct of the Program in Urban, Social and Environmental Policy a Tufts University, and **David Baltimore**, Professor of Biology at MIT.

Krimsky: I am very concerned about the growing influence of industry on academic science. This has existed for many years in the chemical and the nuclear industries. And now it is beginning to occur in the biological sciences, especially with the advent of advances in recombinant DNA.

The direction of research influenced by industry may not be the direction that is in the public interest. Corporate requirements for scientific research may be at odds with pressing societal needs. There are many examples one could cite, especially in environmental research, nutrition and biomedical science.

Our academic institutions and academic scientists need to be free from corporate influence. And such influence can emerge in various ways: through direct funding by corporations to departments and investigators, by scientists serving as consultants to or on advisory boards of industrial firms, or by academic scientists playing a double role by setting up their own small venture-capital firms.

These are dangerous moves if they become widespread because in the end university-based science will lose its sense of detachment. When confronted with policy decisions on the applications of technologies to society we need objective academic involvement. How can the public have confidence in the expert opinion of scientists on the potential risks and benefits of a technology when those same individuals have financial interests in commercial development?

Baltimore: It seems to me that there is nothing wrong with universities serving as a source of technical expertise for newly developing industries or even for mature industries. And that kind of symbiosis between industry and academic departments has been very good for both over history — take the chemical industry.

This seems to be a healthy development for a number of reasons. One is that if there is an industrial use for modern biological techniques, it means that those who are trained in university biological laboratories will have an increased range of employment opportunities. With the recent lateau of hiring into academic positions, it necessary, if we are going to maintain aduate student activity, to find other oportunities for our graduate students.

What's more, we find that after the large investment in basic biological research we are now beginning to get practical spinoffs. While society may value the advancement of abstract knowledge, that is not society's major interest. Consequently, I find it heartening that we can now say to the public, "You've been investing all this money for so long and now things are emerging that are of practical value."

I, too, worry about the academic enterprise being undermined by corporate interests. We can have both an academic focus and industrial involvement but we must maintain a separation between the two. To make certain that university biology departments maintain their autonomy we must be vigilant about a number of things:

- We must be entirely open about industrial affiliations.
- We must maintain a separation between university and industry. When academics consult for industry to help find solutions to industry's problems, they ought to remain consultants and not turn their laboratories into factories for the solution of corporate problems. To be independent, university laboratories must be allowed to follow the internal logic of science.
- We should not use our students for our own ends. Students should continue to have freedom to work out problems that emerge in the laboratory, without being tied to predefined industrial needs.

If we can handle this mixed economy with honesty and integrity, we will have done a service to the university by bringing in new sources of funding and by increasing the importance of the university to industry.

Krimsky: In the 1960s, many scientists (including yourself) expressed moral outrage in learning of the war-related research carried on secretly at many universities. Now we must ask ourselves what the consequences will be if industries ostensibly fund entire departments (as they have done in chemistry, computer sciences, engineering and, more recently, certain areas of policy analysis). Notwithstanding the fact that corporations claim they give with no strings attached, we can [illegible] that they will establish the [illegible] of research. And second, "chilling effect," par[illegible]-funded faculties [illegible]ojects or express [illegible]eted as dis[illegible] corpor[illegible]related

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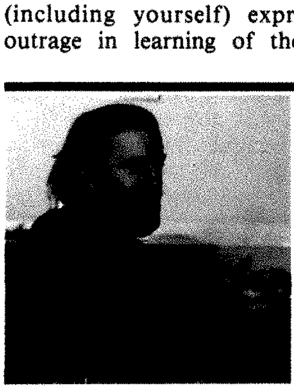
Crucially, during perio[illegible] a about the risks of techno[illegible] independent scientists willing Risks may crop up in research the ultimate marketing of b products. As you know, in the chemical industry we have had ver scientists willing to speak out against potential hazards of petrochemical agent to workers, consumers or military personnel. And now we are faced with the results of their timidity. With so many scientists working directly from the chemical industry, can we expect them to speak freely about the toxic effects of chemicals? How extensive is the influence of industry on biology going to be?

Baltimore: I couldn't agree more. I think it is extremely important that the academic enterprise maintain its freedom to do what it wants, to say what it wants, to be counter to conventional wisdom and to discover things that are counter to the desires of industry.

Nevertheless, the positive side of industrial involvement in the academic world should not be discarded out of fear that university-based scientists will lose their autonomy owing to industrial pressure. We need to maintain a balance between industrial influences and influences from outside the corporate world.

Krimsky: Are there any mechanisms



Krimsky:
'Just as war-related research compromised a generation of scientists, we must anticipate a demise in scientific integrity when corporate funds have an undue influence over academic research'

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available to universities guaranteeing that balance? And as for spin-offs: what role does society play in determining what the research priorities or the practical applications of biological research ought to be? We can have many different kinds of potential spin-offs. Shall we simply allow corporate management to decide autonomously what path to take? Scientists may feel free to choose the fine-structured problems in a research programme at the same time that the shape of the outer boundaries of that programme are exogenously determined. It is in this important respect that science does not develop from some internal logic. I want to see more public and less corporate influence in establishing the outer boundaries.

Baltimore: In the United States the answer to your question is that the desire of people to seek profits determines what research gets turned into useful products for the general economy. If they are not even seen as potentially profitable, they are not pursued or produced. One can argue persuasively that that is not a sufficient reason and that government ought to play a larger development role.

Behind these kinds of disputes stands a naive model: at one side there is a scientific sector with people doing basic research. On the other, independent of basic research scientists, we have the industrial sector, selecting those areas for practical exploitation and deciding how they are to be applied. There is a much more important, neglected relationship. The shape of the outer boundaries of basic research is not merely influenced by the corporate sector alone, either directly or through government, but emerges from research itself. I see the world-wide research effort as a continuum — from the problems determined by the inner logic of science to the problems determined by outside influence.

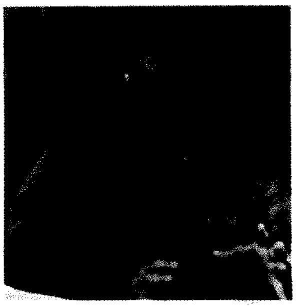
Many scientists work totally out of the logic of their research as it evolves in front of them. For them it doesn't matter what the rest of society thinks about the utility of what they are doing. These are the projects that in the end often turn out to produce the real surprises. No corporation can control them because no-one can see where they are heading.

At the other end of the continuum there are people doing contract research who have been asked to solve specific problems that industry or government wants solved.

Some research is a mixture: scientists get a little money to do something, trying perhaps to cure a disease and at the same time they discover new phenomena. They then have the freedom to explore these new areas.

Krimsky: How are we going to maintain a sufficient number of biologists not tied to the industrial sector, willing to stand up and say: "Wait a minute I am not very confident"? Remember it didn't happen in the petrochemical industry.

Baltimore:
'The positive side of industrial involvement in the academic world should not be discarded out of fear that scientists will lose their antonomy owing to industrial pressure'



Baltimore: Yes, there ought to have been much more questioning of what was going on. Universities should have opened up the debate. The rush of industrial development following World War II undercut the critical function of universities.

We now should look at the strengths of what has happened in the last five to ten years. The environmental movement has been a significant success in its own terms, even though it has not accomplished everything it wanted to. It never will. Nonetheless, it has moved from nowhere to become a major force in our society. That has happened because it has been supported by large groups of people.

Krimsky: But what about the scientific community?

Baltimore: In fact, many of the leaders in these movements have been scientists. Matthew Meselson, for example, is one of the best-recognised scientists in the academic world and yet he is the person who became most identified with opposing defoliation in Vietnam. The movement to stop biological warfare research (which even I had something to do with) came from inside the scientific community, again led by Dr Meselson. The movement to inhibit the development of recombinant DNA research (which, to my mind, got out of hand) came from within the scientific community.

Or consider Bruce Ames, the one person who has really brought a new dimension to our thinking about environmental dangers associated with cancer. He is one of the most eminent scientists in the United States. The Ames test revolutionises our understanding of the dangers inherent in everyday life. This seems to me to be most responsible action, coming directly from the scientific community. Now in fact industry uses the Ames test widely.

Krimsky: But industry has systematically opposed the Ames test as an instrument for establishing policies regarding potential human chemical carcinogens.

What's more, industry has set up its own cadre of scientists and experts to legitimate its opposition to effective testing. The American Industrial Health Council, the American Tobacco Institute and the Chemical Manufacturing Association have all set up private institutes designed to defend their positions and I'm worried that the influence of these institutes will spread into the biological departments of universities.

We do not have sufficient checks and balances. We cannot continue to allow university departments and individual scientists to exercise their own judgements because large amounts of money are likely to be derived from consultancies and from profits from these new biological technologies.

What we need is an independent source of funding, encouraging research on the dangers which might emerge from the new biological technologies. We need to secure the university against undue influence by industry in this new field. There must be a balance between research on developing new technologies and research on the potentially adverse effects of these developments.

Baltimore: I agree that we need to strongly counter attempts by industry to undercut legitimate, validated testing.

Krimsky: One way to accomplish this would be to give incentives to industry to fund universities indirectly through government agencies. Let the government funding agency distribute support to universities rather than allowing corporations to provide direct funding to departments of biology.

But why should industry do this? Clearly, corporations want to be able to direct research at universities and control the purse-strings.

If such funding went through agencies like the National Science Foundation or the National Institutes of Health, peer review committees could distribute the funding to appropriate university-based laboratories. The public would then have some way of overseeing the way the money was spent. We would then not end up with a closed, insulated system.

Baltimore: Where industry directly supports research in a given laboratory, that fact should be on record. One can then see in context the views of a scientist funded by industry when that research worker participates in discussions of work funded by the supporting corporation.

Krimsky: Given that scientists play multiple roles — for example there are those who are faculty members at universities, who also consult for industry and, in addition, sit on government advisory panels — shouldn't these multiple roles b on the public record?

Baltimore: To the extent that it does n unreasonably interfere with a perso s private life, I think it ought to be avail le information. □

NEWS AND VIEWS

Small RNAs and splicing

from Richard Roberts

THE small RNAs found in the nuclei of eukaryotic cells (snRNAs) were first described by Weinberg and Penman more than 10 years ago. They range in size from 90 to 220 nucleotides, are only found in the nucleus and have unusual 5′-terminal structures. These structures resemble the 'cap' structures of eukaryotic mRNAs but contain 2,2,7-trimethylguanosine rather than 7-methylguanosine as the blocking residue. They also contain some additional modified bases notably pseudouridine and 2′-O-methyl nucleosides. Although several (U1, U2, UU3B) have been sequenced by Busch and his colleagues, their function is unknown. This may soon change however as a new experimental attack has been launched which could lead to fresh insights into both the function of these snRNAs and the processing of initial RNA transcripts.

Experiments described in two recent papers (Lerner & Steitz, *Proc. natn. Acad. Sci. U.S.A.* **76**, 5495; 1979 and Lerner *et al.*, this issue of *Nature* page 220) have uncovered an interesting class of subnuclear particles and provide the basis for some fascinating speculation about 'RNA splicing.' The experiments are based on old reports (Mattioli & Reichlin *J. Immunol.* **107**, 1231; 1971; Northway & Tan, *Clin. Immunol. Immunopathol.* **1**, 140; 1972) that certain patients suffering from systemic lupus erythematosus possess circulating antibodies directed against a ribonucleoprotein complex present in the nuclei of many eukaryotic cells. In the first paper, Lerner and Steitz have characterised the RNA and protein components of this complex. They show that the complex contains a specific set of seven polypeptides with molecular weights between 12,000 and 35,000, which are neither histones nor the polypeptides associated with the 30S hnRNP particles. The RNA components are the small nuclear RNAs described above. Different antisera precipitate different subsets of these small RNAs, but always with the same set of seven polypeptides. Furthermore, the experiments show that the snRNAs may be quantitatively precipitated from nuclear extracts in the form of these ribonucleoprotein complexes. The authors propose that each snRNA can interact with the same set of seven polypeptides to form a discrete ribonucleoprotein particle (snRNP).

A further step in the characterisation of the snRNPs is described in this issue of nature (Lerner *et al.*). First it is shown that the lupus antisera precipitate similar snRNP complexes from a variety of eukaryotic sources, ranging from man to insect, indicating that these complexes have been highly conserved during evolution. They are not found, however, in tobacco, yeast, *Dictyostelium discoideum*, or *E. coli* extracts. They are present in highest abundance in metabolically active cells, and from such cells they sediment, in sucrose gradients, with a molecular weight (30S) far greater than that observed (7-10S) for the free snRNP species. This suggests that the 7-10S particles are themselves bound to some other moiety of much higher molecular weight. The authors speculate that the high apparent molecular weight may be due to the attachment, of the snRNP particles, to hnRNA, which is itself associated with 30S hnRNP particles.

The arguments leading to this prediction, while not compelling, are highly intriguing. Several groups have examined the sequences present around the splice junctions in hnRNA and defined a consensus sequence from which any one sequence diverges by only a few nucleotides. This is postulated to be a 'preferred' sequence at which splicing could occur. By examing the sequence of a major snRNA (U1A), the authors noted that its 5′-terminal sequence shows extensive complementarity to this preferred sequence. Nucleotides 3 to 8 from U1A exactly match that part of the 'preferred' sequence found at the 5′ end of the intervening sequence, while nucleotides 9 to 11 exactly match the 3′ end of the intervening sequence. Some further possibilities for base pairing exist between nucleotides 12 to 20 of U1A RNA and the continuation of the 3′ intervening sequence. Thus it is hypothesised that the 5′-terminal sequences of U1A RNA interact with the terminal sequences of the intervening regions and thus serve a template function by correctly aligning the sequences to be spliced. Consistent with this role for the 5′-terminal sequences of U1A RNA is the finding that among the snRNP particles is a minority class which does not show the high apparent molecular weight in sucrose density gradients. This class contains a shortened form of U1A RNA which is missing both the 5′ terminal 'cap' structure and the first six nucleotides from the 5′ end. The absence of these nucleotides would seriously disrupt the fit required by the model. It is not known whether these particles, containing the shortened form of U1A RNA, are an artefact of isolation or have metabolic significance.

A similar scheme has previously been proposed in the adenovirus-2 (Ad2) system, this time invoking the Ad2 encoded VA RNA as a template for the processing of late Ad2 mRNAs (L. Philipson, unpublished *News & Views* **277**,593; 1979; Murray & Holliday FEBS *Lett.* **106**, 5; 1979). Here, the detailed model of Murray and Holliday seems less attractive since it requires base pairing with nucleotides which lie beyond the known 3′ end of VA RNA. Whatever the final outcome of these predictions, it seems that at last an experimental system is available for the study of RNA processing events within the eukaryotic nucleus and such studies should be spurred by the tantalising hypothesis developed in this latest report.

Amidst the frenzy of activity presently aimed at cloning and sequencing DNA, we should perhaps remember that many of the highly conserved processes of molecular biology involve only RNA and protein. The specificity of protein synthesis is provided by the triplet interaction between tRNA and mRNA, while its initiation requires RNA-RNA interaction between the 3′-end of ribosomal RNA and mRNA. The ribosome which catalyses this process contains both RNA and protein and recently an RNA processing enzyme,

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Richard Roberts is a Senior Staff Investigator at the Cold Spring Harbor Laboratory, Long Island, New York.

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RNase P of *E. coli,* has also been shown to consist of both RNA and protein (Stark *et al. Proc. natn. Acad. Sci. U.S.A.* **75,** 3717; 1978). Some of us would argue that this reflects the early establishment of these processes during evolution, at a time preceding the arrival of DNA on the biological scene. If RNA splicing is also a process which developed at an early stage in evolution (*News & Views* **272,** 581; 1978; Darnell, *Science* **202,** 1257; 1978) then it may not prove surprising if its specificity also depends upon RNA-RNA interactions. ☐

Galaxies: the origin of spiral structure

from M.G. Edmunds

A RECENT comparison of the morphology and rotational properties of nearby galaxies, together with computer simulations of a simple model of patterns of star formation, may have greatly clarified our understanding of the causes of the beautiful spiral structures seen in many large systems. The origin of such structure remained a source of speculation for many years until during the 1960s the formulation of a 'density wave' theory seemed to provide a suitable physical mechanism. A clue lay in the fact that well-developed spiral structure is seen only in galaxies with disks containing both stars and gas. The basis of the theory is the propagation around the centre of the galaxy of a perturbation in the space density of stars. The gas in the interstellar medium responds strongly to the perturbation in the gravitational field, and its compression results in a shock wave which instigates the gravitational collapse of gas and dust to form stars. It is the very non-linear behaviour of the gas which gives rise to the large influence on the overall appearance of a galaxy for only a small perturbation in the underlying distribution of stars. Of the stars which form, the massive ones are extremely luminous, and it is they which are seen delineating the spiral pattern. Such stars are short lived and soon die out, leaving the wave to show itself by new star formation as it moves relative to the interstellar gas. The pattern of the wave rotates 'rigidly' around the galaxy's centre, although the gas in stars may be in differential rotation — and hence relative shearing motion. The extent of the differential rotation is governed by the overall gravitational field experienced by the stars and gas particles.

Unfortunately a fundamental difficulty arose in understanding how the spiral wave manages to maintain itself against dissipation. A particularly important dissipating process will occur at the so-called 'inner Lindblad resonance'. A star in a galaxy will have a bounded orbit around the centre, but the orbit will not normally be either circular or closed. The intervals between the star being at some particular distance from the centre will, however, be well defined with the frequency of successive inward or outward passages being called the 'epicyclic' frequency, and this is determined by the local gravitational field. Now if the frequency of successive passages past the star of the density wave perturbation in gravitational field exactly matches the epicyclic frequency at that radius, then the star will experience acceleration due to the perturbation at the same point on successive orbits. A resonance will hence occur which will feed energy out of the density wave and into the velocities of the resonated stars. The problem for the density-wave theory is to replace this loss of energy, and no obvious method exists for a single isolated galaxy with a simple pure spiral pattern. A way round the difficulty would be to show that isolated spiral galaxies which show good spiral structure do not have inner Lindblad resonances. No resonance would occur if there were no differential rotation, since the pattern speed in this case will never match the epicyclic frequency.

Such behaviour has indeed been found by J. Kormendy and C.A. Norman (*Astrophys. J.* **233,** 539; 1979) in a discussion of the morphology of some 54 spiral galaxies for which rotation velocities are available. Those galaxies which are on their own and have well-developed spiral patterns do not differentially rotate over the region where the spiral structure is apparent. The galaxies must be able to find enough energy transfer to overcome any other (less important) dissipation of the wave. But these galaxies represent only one-fifth of the sample. Over half the sample do differentially rotate and hence are expected to have an inner Lindblad resonance. Yet they still show good spiral structure, the difference being that the differential rotators either have a nearby companion galaxy or else have a bar structure in their centres. An example of a barred galaxy is shown in Fig. 1, and a less

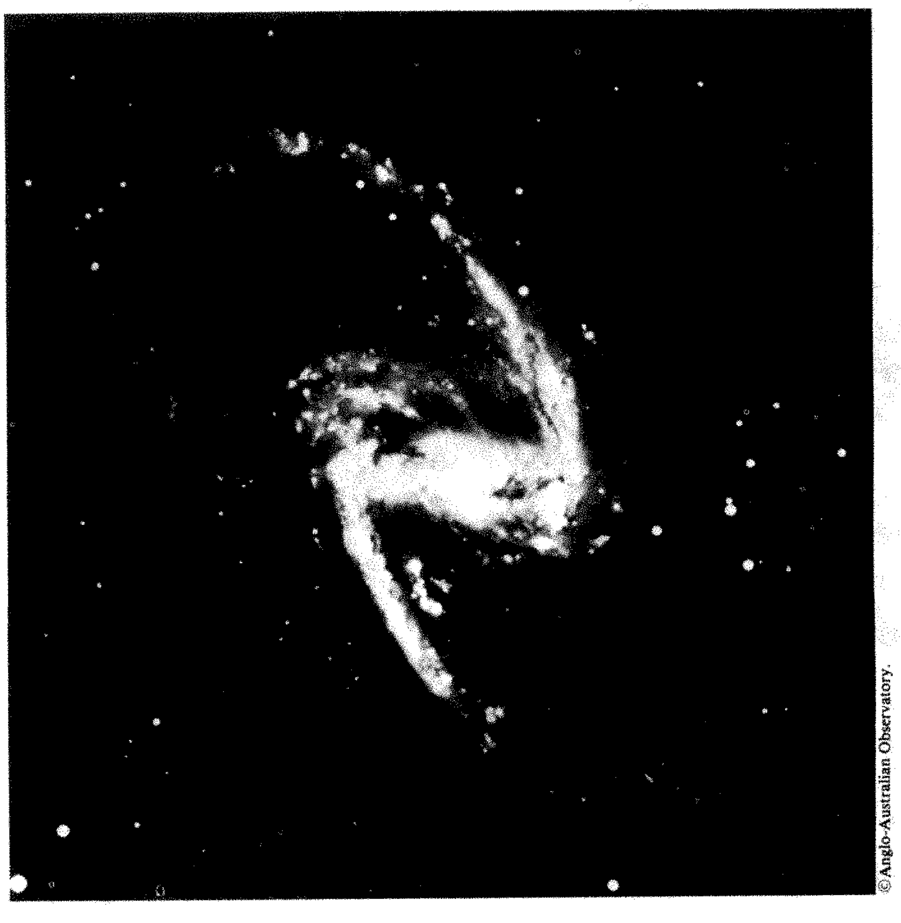

Fig. 1. NGC 1365 showing a well-developed bar and two-armed spiral structure.

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M.G. Edmunds is a lecturer in the Department of Applied Mathematics and Astronomy, University College, Cardiff.

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extreme (but still barred) case in Fig.2. The importance of the companion or bar is in providing a significant rotating non-axisymmetric gravitational potential which will 'stir' the galaxy and force the spiral structure. The pattern is still the result of a density wave, but the driving of the wave will presumably replenish the energy lost at the inner Lindblad resonance. The initial formation of the bar presents no problems, since numerical simulations of the stellar dynamics in galaxies show a particular willingness to evolve rapidly to give a central bar.

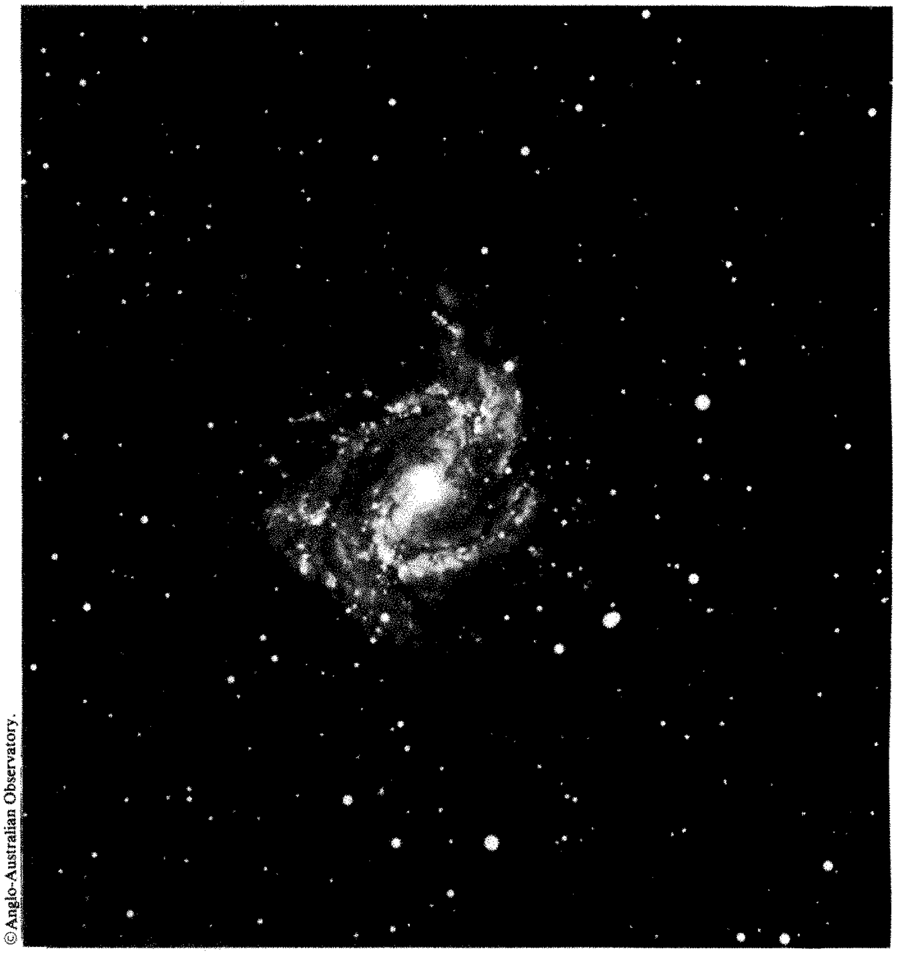
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Fig.2. NGC 5236 (M 83) showing a less obvious, but still important, central bar.

There still remains a significant fraction of galaxies in the sample which differentially rotate, but have neither bar nor companion. An example of the morphology of this type of galaxy (although not one from Kormendy and Norman's sample) is shown in Fig.3. The spiral structure is very fragmentary, with no grand two-armed design, but rather a system of multiple and branched arms which can only be individually followed over short distances. P.E. Seiden and H. Gerola (*Astrophys. J.* **233**, 56; 1979) have developed a series of computer models which nicely simulate such structures, and show that it may have very little at all to do with a global density wave pattern. Their model supposes that the supernova explosion of the bright massive stars which illuminate spiral structure can produce further star formation, since the shock which the explosion pushes out into the interstellar medium may induce the collapse of nearby interstellar clouds. The new massive stars formed would subsequently also explode, and so the star formation would propagate through the galaxy. Strong differential rotation of the disk would tend to pull such regions of star formation out into an arc — causing a short spiral arm. An element of randomness is introduced by allowing some spontaneous star formation, and the correlated activity which gives rise to structure is produced by having a higher probability for the supernova-induced star formation. Since there is some evidence from supernova remnants in our own Galaxy that such induced star formation can occur, Seiden and Gerola's model is attractive, and must also play some part in influencing the morphology of spiral arms even when a large-scale density wave is operative.

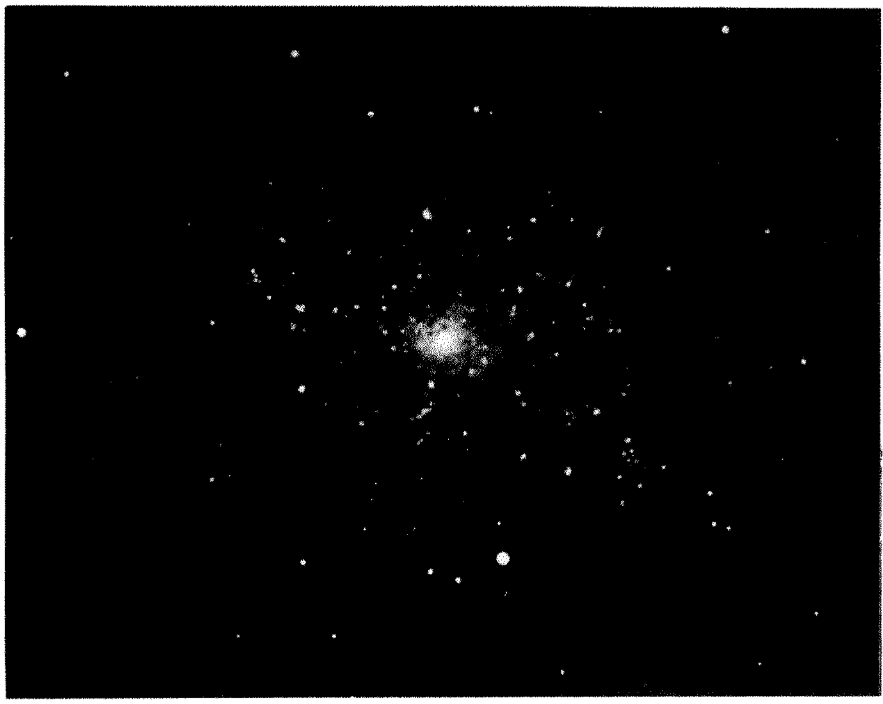
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Fig.3. NGC 7793 showing multi-armed, filamentary spiral structure.

Life would not be so interesting without exceptions, and two unusual galaxies turn up in Kormendy and Norman's sample. These are both isolated single-arm (as opposed to normal double-arm) and they differentially rotate, although the spiral arm in one of them (NGC 4378) can be traced over almost 1¼ revolutions. But the fact that only two cases out of a sample of 54 cannot be immediately classified is surely encouraging.

There remain lingering theoretical problems connected with preventing a stellar disk from becoming too unstable to bar formation without either an unacceptably large velocity dispersion among the stars or a rather *ad hoc* massive halo of low luminosity objects surrounding the disk. Despite this, the three mechanisms — a self-sustaining two-armed density wave in the absence of an inner Lindblad resonance, a density wave driven by the gravitational perturbation from a stellar bar or companion galaxy, or stochastic supernova-induced star formation with differential shear in the disk — seem to provide a reasonable basis on which to account for the structure of spiral galaxies. □

Carcinogens and DNA

from Stephen Neidle

THE physical and chemical properties of the potent diolepoxide carcinogens have been much studied in the hope of determining the molecular basis of their carcinogenic and mutagenic effects. They are known to bind directly to DNA, and this reaction has formed the focus of both experimental and theoretical investigations into the activity of diolepoxides such as benzo(*a*)pyrene 7,8 dihydrodiol 9,10 oxide, the most important active carcinogenic metabolite of benzo(*a*)pyrene.

Although theoretical approaches to the likely conformation and chemical reactivity of the four possible stereoisomers of benzo(*a*)pyrene diolepoxide, using quantum-mechanical methodology, have had great influence on thinking in this field, in many cases the theoretical findings conflict with the experimental evidence and it is fair to say that only some of the recent theoretical treatments are successful or other than trivial in their predictions. The deficiencies and limitations of some approaches are highlighted for example, by their inability to explain satisfactorily the considerably greater tumorigenicity and DNA binding ability of the *anti* compared with the *syn* isomer (Brookes *Cancer Lett.* **6,** 285; 1979), even though the theoretically-derived data indicate that the *syn* isomer is the more stable (Klopman, Grinberg & Hopfinger *J. theor. Biol.* **79,** 355; 1979) and until relatively recently was believed to be the biologically significant one.

Application of the ϕX174 DNA assay system developed by Harvey and coworkers to a series of diolepoxide carcinogens, has led to the revelation of further deficiencies in some quantum mechanical analyses (Hsu *et al. Biochem. biophys. Res. Commun.* **87,** 416; **88,** 1; 1979). This assay system is remarkably effective in correlating inhibition of viral infectivity with carcinogenic potency of the various diolepoxides, yet this correlation was not followed either by predictions of reactivities from molecular orbital theory or by the relative structure positions of the diolepoxide groupings on the hydrocarbon skeletons.

Clearly, studies of the chemicals themselves are not enough to explain their different carcinogenic potencies and other factors such as the geometry and kinetics of their binding to the DNA receptor sites will have to be taken into account.

Benzo(*a*)pyrene diolepoxide carcinogenesis is generally considered to involve direct binding to DNA, with guanine residues as the major sites of attachment at least in terms of stoichiometry. About 10% of the total binding is to adenine however, and although its significance is unclear, some recent evidence correlates binding to adenines and not to guanines with mouse skin tumorigenicity (Di Giovanni *et al. Cancer Lett.* **7,** 39; 1979), although in this report no adducts have actually been characterised as diolepoxide ones. Adenine-modified regions of DNA are known to have rather different conformational properties in terms of greater local denaturation than their guanine counterparts. The *anti* form of the diol epoxide can be resolved to two stereoisomers. Their interactions with DNA are, not surprisingly, stereospecific (Meehan & Straub *Nature* **277,** 410; 1979), with essentially only the (+) isomer binding to double-stranded DNA (to guanines) whereas denatured, single-stranded DNA was equally susceptible to both isomers. These differences in binding ability correlate with biological activity in several cell lines — the (+) isomer being more carcinogenic — and Meehan and Straub suggest that DNA secondary structure is an important determinant in carcinogenicity.

One question of interest in looking at the binding of carcinogens to DNA is whether any specific base sequence is involved. Weinstein, Grunberger and coworkers (*Biochemistry,* in the press) do to a large extent confirm Meehan & Straub's findings, with the additional important observation from restriction enzyme analysis of modified SV40 and lambda DNA, that no appreciable specificity of binding in respect of base sequence was apparent. However, subtle and possibly important effects of binding specificity may occur. Probably more crucial to carcinogenicity is the geometry of the binding in relation to subsequent excision repair by endonucleases. Binding to different residues and different chemical groups within a residue dramatically affects excisability. Increased excisability implies attachment on the outside rather than to deeply buried sites within the molecule.

There is no lack of molecular models for the structure of benzo(*a*)pyrene diol epoxide-modified DNA, some of which owe more to imagination than to reality. The recent suggestions of Beland (*Chem.-Biol. Interactions* **22,** 329; 1978) are among the more plausible and detailed with the benzo(*a*)pyrene chromophore being situated in the DNA minor groove and causing some local denaturation of the double helix. This general type of non-intercalative model is strongly supported by several recent physical measurements on the diol epoxide–DNA complex. Fluorescence probe techniques (Prusik *et al. Photochem. Photobiol.* **29,** 223; 1979; Prusik & Geacintov *Biochem. biophys. Res. Commun.* **88,** 782; 1979) give data consistent with the benzo(*a*)pyrene chromophore attached (by N2 of guanine) externally to the DNA double helix, and oriented with at most a 35° angle to the helix axis. In particular, intercalation (at least in the sense of the classical Lerman model) is ruled out by these workers on account of the accessibility of the chromophore to external probings. Additional evidence for a non-intercalative model is also provided by the new technique of optically-detected magnetic resonance (Lefkowitz *et al. FEBS Lett.* **105,** 77; 1979)

Unfortunately such techniques cannot provide detailed information on the molecular geometry of the modified residues of DNA. The situation is made yet more complex by the growing realisation that DNA structure is not always necessarily of the classical forms. There is now powerful evidence for example, that tracts of alternating pyrimidine-purine residues have highly unusual structures (see for example *Nature* **282,** 680; 1979; *News and Views,* **283,** 11; 1980); it would not be surprising if binding of carcinogens did not sometimes exploit such peculiarities. Molecular model-building of nucleic acid-carcinogen interaction urgently requires input of firm data from the fine structure techniques of X-ray crystallography and NMR in order to achieve credibility. Questions of the relevance of the structural peculiarities mentioned above to differences in excisability and repair, and to carcinogenic and mutagenic processes in general must await future investigations. ☐

Stephen Neidle is a Career Development Awardee of the Cancer Research Campaign, working in the Department of Biophysics, King's College, London.

The specificity of plant defences

from R.A. Dixon & C.J. Lamb

WHEN plants are attacked by bacterial or fungal pathogens they synthesise and accumulate nonspecific antimicrobial compounds known as phytoalexins. A few years ago it was established that phytoalexins could be induced to accumulate by glucan and glycoprotein 'elicitor' molecules associated with the cell wall of the invading pathogen (see *News & Views* **273,** 266; 1978) and this has started a search for the molecular basis of the exquisite specificity seen in the classical 'gene-for-gene' relationship between plant cultivars and physiological races of a pathogen, looked at in terms of elicitors and phytoalexin production.

In the simplest terms the gene-for-gene relationship consists of a gene for 'avirulence' in the pathogen being complemented by a specific one for resistance in the plant — the plant and pathogen are in this case incompatible.

R.A. Dixon is in the Department of Biochemistry, Royal Holloway College, University of London and C.J. Lamb is in the Department of Biochemistry, University of Oxford.

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Disease results when a mutation in the pathogen produces a new avirulence gene (now a 'virulence' gene) that is not matched by the available resistance genes carried by the host cultivar — the plant and pathogen are now compatible. Plant breeders have long been familiar with the ability of plant pathogens to keep one jump ahead by continually throwing up new races to which hitherto resistant varieties of crops are completely susceptible.

The simplest model for the gene-for-gene phenomenon in terms of elicitors requires that a race-specific elicitor (presumably the product of the pathogen's avirulence gene) is recognised by a specific receptor on the plant cell (presumed to be the product of the plant's resistance gene); phytoalexin production is induced and the pathogen is limited. If a mutation either in the pathogen or plant means that the elicitor is no longer recognised by the plant, then the infection is not limited and disease follows. The key predictions of this model are first, the existence of race-specific elicitors, and second, that the differential accumulation of phytoalexin is the most important determinant of disease resistance.

Recent work suggests that this model is indeed an oversimplification and that the real world is considerably more complex. On the question of race-specific elicitors Keen *et al.* (*Physiol. Plant Pathol.* **15,** 43; 1979) recently claimed to show race-specific elicitors in the cell envelopes of physiological races of the bacterium *Pseudomonas glycinea*, which causes bacterial blight in soybeans. High molecular weight glycoproteins solubilised by detergent treatment showed the same specificity, with one exception, in eliciting accumulation of the phytoalexin glyceollin in cotyledons of two varieties of soybean as did the bacterial races from which the elicitors were isolated.

In contrast, however, Albersheim and coworkers (*Plant Physiol.* **57,** 760; 1976) have shown that glucan elicitors from four different races of the fungal pathogen *Phytophthora megasperma* var. *sojae* (Pms) are qualitatively and quantitatively identical suggesting that further 'specificity factors' are needed to account for race-specific resistance. A recent report by Wade and Albersheim (*Proc. natn. Acad. Sci. U.S.A.* **76,** 4433; 1979) now identifies glycopeptides secreted into the culture medium by Pms as the proposed factors. Glycopeptides from three races of Pms were tested for their ability to prevent the expression of serious symptoms in four soybean cultivars. Glycopeptides from incompatible races of the pathogen but not from compatible ones protected seedlings from attack by compatible races. Glycopeptides from compatible races did not cause cultivars resistant to a given Pms race to become susceptible to that race. The glycopeptides themselves are only weak elicitors and their effect on glucan elicitor action remains to be established. Wade and Albersheim postulate that synthesis of the glycopeptides is controlled by the pathogen's avirulence genes and that in incompatible but not compatible interactions they trigger a defence reaction which decreases the rate of pathogen development and enables inhibitory levels of phytoalexin to accumulate in the area of initial fungal penetration.

Such a hypothesis still leaves open the nature of the proposed defence reaction and the product of the host's resistance gene. This model assumes no determinative role for elicitor-induced phytoalexin accumulation in the soybean-Pms system and indeed Albersheim and coworkers (*Plant Physiol.* **57,** 760; 1976) have shown that initial rates of glyceollin accumulation are identical during infection of wounded hypocotyls by compatible and incompatible races of Pms. This contradicts earlier work of Keen (*Physiol. Plant Pathol.* **1,** 165; 1971) who observed differential rates of glyceollin accumulation in this system. However, in both studies no account is taken of the cellular and subcellular distribution of glyceollin and such considerations may be crucial for a full understanding of the role of phytoalexins in race-specific resistance.

A common feature of the models of Keen and Albersheim is that specificity is associated with the incompatible interaction. However, this may not always be the case. Two recent papers by Kuć and coworkers (*Physiol. Plant Pathol.* **15,** 117 and 127; 1979) suggest that in potato cultivars with R genes, specificity in resistance to physiological races of *Phytophthora infestans*, the causal agent of potato blight, is associated with compatibility, and a phytoalexin suppressor, or 'hypersensitivity inhibiting factor' is proposed as the determinant of specificity. They found that mycelia and zoospores of a compatible race of *P. infestans* contain β, 1-3, β, 1-6-linked low molecular weight glucans which could inhibit the rapid cell death, loss of electrolytes, tissue browning and accumulation of terpenoid phytoalexins associated with the hypersensitive reaction of potato tissues to treatment either with elicitor from the same compatible race or infection by an incompatible race. Although the incompatible race contained similar glucans they were much less active as suppressors of the hypersensitive reaction.

The glucans consisted of non-anionic and anionic fractions, the latter containing a small proportion of glucose residues as phosphoryl monoesters. In preliminary experiments, mixing elicitors from compatible or incompatible races with a membrane preparation from potato tubers led to decreased elicitor activity on subsequent application to tuber tissues, possibly as a result of sequestration of elicitors by binding sites on the membrane preparation. Only glucans from the compatible race were able to restore elicitor activity, suggesting that the small glucans may compete for the elicitor binding sites. These molecules may prove valuable tools in the search for elicitor receptor sites. Elicitors from *P. infestans* agglutinate potato protoplasts indicating cell surface binding sites (*Science* **201,** 364; 1978), but it is not clear whether these sites are receptors for elicitor induction of phytoalexin accumulation.

Although no unified hypothesis has yet emerged, the demonstration of molecules that determine specificity in plant–pathogen interactions is a major development in the study of the molecular basis of race-specific disease resistance. Clearly there is considerable scope for collaboration between biochemists and pathologists in this important field. □

Patterns of ungulate reproduction

from Robert M. May

ONE theme, around which much current ecological research is organised, concerns the relation between an animal's physical and biological environment (its habitat and the species it interacts with) and its foraging and reproductive behaviour. In this context, Robbins and Robbins (*Am. Nat.* **114,** 101; 1979) have recently drawn together a lot of data on ungulate and subungulate species, with a view to elucidating fetal and neonatal growth patterns, and trends in maternal reproductive effort. These authors believe ungulates to be especially interesting, because they inhabit a wide range of environments, and comprise species in many different stages of domestication.

By plotting the weight of the fetus (expressed as a fraction of the weight at parturition) as a function of time (expressed as a fraction of the gestation period) for several species, Robbins and Robbins show the fetal growth rate in ungulates is a power function that varies little among species, and is typically of the general mammalian pattern derived by Sacher and Staffeldt (*Am. Nat.* **108,** 593; 1974) and others. Using data for white-tailed deer, domestic sheep and cattle, they also show the weight of the actual fetus, as a percentage of the gravid uterus, increases steadily throughout gestation, to around 60% at parturition. The pattern shows no significant differences between natural and domesticated ungulates; that is, selection for a larger neonate in domesticated animals entails a proportional enlargement of the uterus, fluids and membranes. As Robbins and Robbins point out, this fact

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Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

suggests "The efficiency of the reproductive process in terms of fetal energy or matter produced per unit of uterine tissue may already be selectively maximized" in natural populations. Similarly, under maternal nursing the maximum growth rates of newly born animals ('neonates') of different species increase in a systematic fashion with increasing maternal weight, with no significant differences between natural and domesticated ungulates. Estimates of the average milk production at the peak of lactation by well-nourished females, nursing a single neonate, also reflect no more than a systematic, curvilinear increase with increasing maternal weight.

The relationship between weight of offspring at birth (expressed as a percentage of maternal weight, or 'proportionate birth weight') and average maternal weight is shown in Fig. 1, for some 69 species. We see the maximum relative reproductive effort apparently occurs in pronghorn antelope and springbok, at around 15%, and decreases to around 4–5% for the very large ungulates and subungulates. The trend to diminishing proportionate birth weight with increasing maternal weight is fairly steady for the larger animals, but there is much variability among the small species (adult weights less than or around 100 kg). Although there is too much scatter for any crisp generalisation, Fig. 1 shows that several north temperate and arctic ungulates (for example, caribou, muskox, bighorn sheep) tend to produce relatively smaller offspring than most African ungulates, with North American species typically being intermediate. The explanation for the relatively small single fetus of caribou, muskox and bighorn sheep may lie in "Inadequate food supplies during winter and the need to optimize maternal survival relative to reproductive effort"; these factors apparently override thermodynamic considerations, which would argue for the largest possible neonate, to minimise heat loss in a cold environment.

As Robbins and Robbins emphasise, there are yet further complications among the smaller species represented in Fig. 1. The Mexican red brocket deer, a shy tropical animal which has one of the lowest proportionate birth weights of the smaller ruminants, has a postpartum estrus. So do muntjac deer and several African bovids. These species live in relatively stable environments, and the combination of relatively low birth weight and postpartum estrus enables them to distribute reproduction throughout the year, in a way that may well increase total reproductive output.

In short, Robbins and Robbins' comparisons among species of ungulates and subungulates suggest that "very basic controlling mechanisms" may produce systematic patterns in fetal and neonatal growth, with these patterns being common to natural and domesticated species. The proportionate birth weights of the smaller species, however, tend to exhibit a fascinating richness of detail that overwhelms any easy generalisation. □

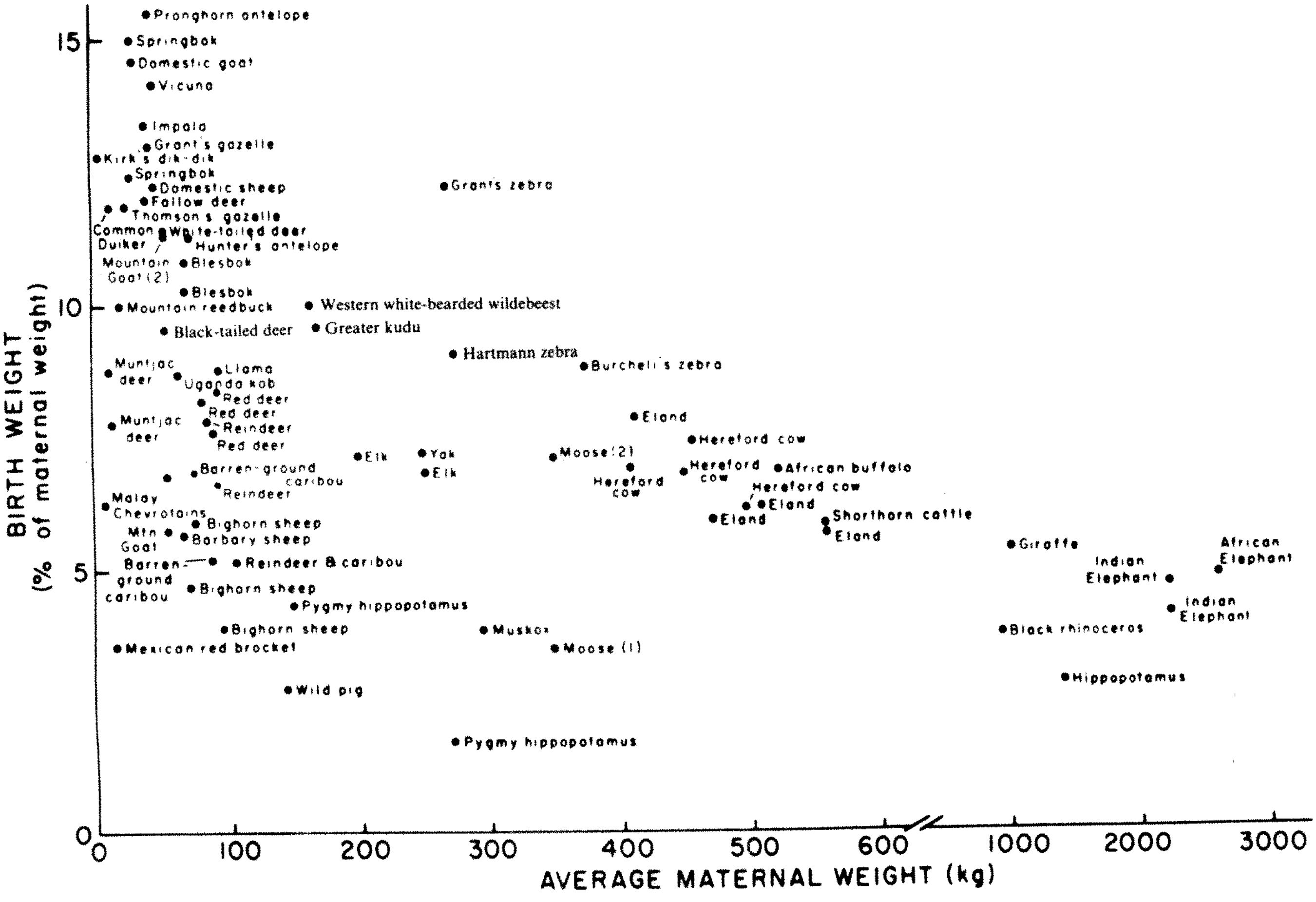

Fig. 1 Birth weight (as a fraction of maternal weight) as a function of average maternal weight in ungulate and subungulate species.

The non-linear spiral

from J. David Pye

THE sense organ of the ear is the cochlea which, as its name implies, is shaped like a snail's shell. Inside its hard, bony wall are three liquid-filled chambers that run together round the spiral from the base to the tip. One of the dividing partitions is a very thin, acoustically transparent membrane but the other, the basilar membrane, is an elaborate structure. It bears four rows of sensory hair cells that are connected by synapses to the ends of nerve fibres that run to the brain.

Sound-induced vibrations of the eardrum are efficiently transmitted to the incompressible cochlear liquids by a chain of three tiny bones. A relatively slow travelling wave is then initiated in the basilar membrane and its movement excites the hair cells. For high-pitched tones the travelling wave builds up quickly

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to a peak near the base of the cochlea and then decays while for deeper tones it runs further towards the tip, thus exciting a different population of hair cells.

The cochlea therefore, not only transduces sound energy into nerve signals to the brain but also acts as a mechanical frequency analyser. But despite extensive study, neither of these functions is well understood in detail, for the known properties have so far not yielded a satisfactory synthesis. New findings from several sources now suggest that some parts of the mechanical action are non-linear and this realisation has greatly stimulated both theoretical speculation and new kinds of observations.

The new developments were aired at a recent meeting in London* and some of the highlights are mentioned below. Non-linearity implies that a response is not directly proportional to its cause (the response curve is not straight); it also leads to the local generation of distortion products that are not present in the original stimulus. This is of course a common feature of any system that is 'overdriven' by excessive stimuli and indeed the beat-notes and other combination tones produced in the ear by loud sounds are already familiar. It is surprising, however, to find that the ear is markedly non-linear even at moderate and low sound intensities and that this property is related to active processes in the living organ.

Conventional linear models of cochlear mechanics have always presented problems and non-linear models which embrace some previously unexplained phenomena have been proposed. Indirect evidence of non-linear motion has been obtained by electrophysiological and acoustic techniques (D. O. Kim, Washington University).

Direct evidence of non-linearity has been provided by E. Lepage and B. Johnstone of the University of Western Australia, Perth, who measured movements of the cochlear partition in guinea pigs by a micro-capacitative probe. They were able to confirm the results of W.S. Rhode of the University of Wisconsin, Madison who had previously demonstrated non-linearity in monkeys by a less sensitive Mössbauer technique.

Further direct evidence was provided by D.T. Kemp (The Royal National Hospital, London) who discussed in detail his newly discovered mechanical evoked response or 'cochlear echo'. He and his collaborators have shown that brief sounds introduced into the ear canal can be detected again several milliseconds later as a vibration of the eardrum coming out from the cochlea. This is thought to occur because active non-linearity in the mechanical action of the basilar membrane would produce local changes in its impedance from which some of the energy will be reflected back towards the base of the cochlea. The involvement of active mechanical processes, using energy derived from physiological sources, was unexpected; but measurements of the echo show that under certain conditions it may contain more energy than is available from a passive reflection from that point. The theories of passive non-linearity that originally predicted the effect have therefore had to be revised.

The phenomenon has been independently confirmed by other workers (J.P. Wilson, University of Keele; H.P. Wit & R.J. Ritsma, University Hospital; Groningen; W.L. Rutten, Academic Hospital, Leiden; Kim). As the cochlear 'Kemp echo' has been shown to depend on normal physiological functioning of the living cochlea, its measurement can form a valuable new diagnostic tool. It is also related to the fine structure of the normal hearing curve (which is not smooth but shows regular, very sharply tuned peaks of up to 10 dB) and both responses can be detected by instruments at sound levels far below the audible threshold (Wilson). That the echo originates from mechanical non-linearity is further demonstrated by its relationship to the distortion products, especially the difference tone (f_2-f_1) and the cubic term ($2f_1$-f_2). Finally, it seems to be intimately linked with certain forms of tinnitus, where the cochlea oscillates spontaneously (for zero stimulus energy) and produces an objective tone that can be recorded from the eardrum.

In the field of psychoacoustic and electrophysiological observations, particular attention is being devoted to the physiologically vulnerable combination tones (f_2-f_1 and $2f_1$-f_2) and to the elusive 'second filter' that makes the responses of single nerve fibres more sharply tuned than the basilar membrane that excites them. Here again the properties of the cochlear echo (in response to paired stimulations) may provide a valuable new kind of evidence. But A.R. Palmer and E.F. Evans (University of Keele) found that single unit recordings from the acoustic nerve gave no evidence of mechanical non-linearity in the cochlea.

In discussions of cochlear mechanisms, of especial interest was the demonstration by A. Flock (Karolinska Institute, Stockholm) that the simple stereocilia of the hair cells are filled with actin filaments which also occur in the cuticular plate and round the apical circumference of the cell itself. The discovery of contractile proteins at these sites could be of great significance in understanding the transduction from mechanical to nervous (that is, electrical) energy and the part played by active, non-linear mechanical processes.

A.C. Crawford and R. Fettiplace (Cambridge University) showed that electrical stimulation of hair cells in the terrapin produces a finely tuned electrical response similar to that elicited by sound. The very short cochlea in the terrapin is probably an ineffective mechanical analyser and this discovery suggests that the second filter involves cellular processes. In the more traditional guinea pig, I.J. Russell and P.M. Sellick (University of Sussex) and Y. Tanaka (Dokkyo University, Tichigi) and his colleagues showed that the inner and outer rows of hair cells have very different response properties that may contribute separately to the final output of the cochlea.

It seems safe to predict that this meeting will come to be regarded as a milestone in the study of hearing. Hi-fi enthusiasts may be shocked, but non-linearity of the normal, healthy cochlea could be a key to its amazing performance. ☐

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*A meeting on non-linear and active mechanical processes in the cochlea was held on 17-19 September, 1979 at the Institute of Laryngology and Otology of the Royal National Throat Nose and Ear Hospital, London. It was organised by Dr D.T. Kemp and Mr S.D. Anderson.

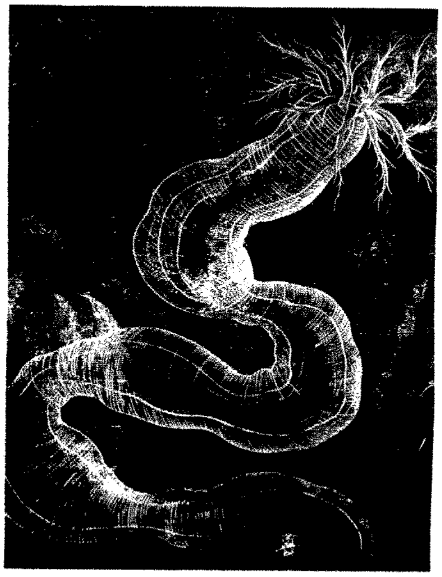
J. David Pye is Professor in the Department of Zoology and Comparative Physiology, Queen Mary College, London.

100 years ago

We pass on therefore to the lower marine animals, and select as an example of the way they are treated a rare British species allied to the sea cucumbers, and named *Synapta duvernea*. It was discovered in the English Channel by M. Quatre-fages, who thus describes it:-

"Imagine a cylinder of rose-coloured crystal as much as eighteen inches long and more than an inch in diameter, traversed in all its length by five narrow ribbands of white silk, and its head surmounted by a living flower whose twelve tentacles of purest white fall behind in a graceful curve.

From *Nature* **21**, 8 Jan., 234; 1880.



REVIEW ARTICLE

The pathogenesis of cancer metastasis

George Poste
Department of Experimental Pathology, Roswell Park Memorial Institute, Buffalo, New York 14263
Isaiah J. Fidler
Cancer Biology Program, National Cancer Institute, Frederick Cancer Research Center, Frederick, Maryland 21701

Metastases do not result from random survival of cells released from the primary tumour but from the selective growth of specialised subpopulations of highly metastatic cells endowed with specific properties that befit them to complete each step of the metastatic process.

UNDERSTANDING how malignant tumour cells are able to spread from the primary tumour to form secondary tumours (metastases) at other sites in the body is one of the major goals of cancer research. Despite advances in surgical technique and the development of aggressive adjuvant therapies for treatment of primary neoplasms, most deaths in cancer patients are caused by metastases. Research done in many laboratories in the last few years has provided important new information about basic events in the pathogenesis of metastasis and the identity of the tumour cells responsible for this fatal disease. A number of these observations challenge long held views and raise serious doubts about the adequacy of certain experimental approaches used to date to study the malignant cell. This article reviews some of these findings and their implications for the experimental analysis of the malignant phenotype and the development of agents for treatment of metastatic disease.

The metastatic process

A metastasis is a neoplastic lesion that arises from another cancer with which it is no longer in contiguity. The ability to produce metastases is a property of malignant tumour cells. Malignancy is not a characteristic shared by all tumour cells and cells that lack the ability to invade and metastasise are termed benign. This classification may seem too obvious for inclusion in a review article on metastasis. However, the most cursory examination of the literature reveals that the term 'malignant' is being used increasingly, albeit incorrectly, as a synonym for 'neoplastic' or 'tumourigenic'. It is also common to see publications that erroneously refer to '*malignant* transformation' of cells *in vitro*. In far too many instances this description is based on inadequate criteria such as changes in cell growth *in vitro* or biochemical alterations and experiments to determine whether such cells can cause tumours *in vivo* (that is, neoplastic transformation) yet alone produce metastases (that is, malignant transformation) have not been done. In this article the term 'malignant' refers only to cells that are capable of producing metastases.

The process of metastasis involves a series of sequential steps[1–3] in which malignant cells are released from the primary tumour and disseminate to distant sites where they proliferate to form new tumour foci (Fig. 1). Metastasis begins with the local invasion of the surrounding host tissue by cells from the primary tumour, either singly, or as cell clumps, or as large multicellular tongues[1]. It is not known exactly how malignant cells invade host tissues. Active and/or passive movements of tumour cells into host tissues are probably important[1]. The ability of some malignant cells to release tissue-destructive enzymes, particularly lysosomal hydrolases and collagenolytic enzymes, has also attracted attention as a possible factor in promoting tumour invasion[6–12]. However, as with virtually all aspects of tumour biology, generalisation is difficult and malignant cell lines exhibit a broad range of tissue digestive capacities[6–10].

In certain neoplasms, distant tumour foci are established by a direct spread of cells from the primary lesion along preformed mechanical pathways such as fascia planes and nerve sheaths[2,12]. Similarly, for tumours growing in major body cavities, shedding of cells from the primary lesion can produce secondary growths by seeding of cells onto the mucosal and/or serosal surfaces of other organs[12].

The more common, and most important, route of tumour spread involves invasion and penetration of tumour cells into blood vessels and/or lymphatics, with their subsequent dissemination to distant organs in the blood or lymph. Lymphatics and small, thin-walled venules offer relatively little mechanical resistance to penetration by tumour cells[2–5]. In contrast, arteries and arterioles are rarely invaded[5]. Blood vessels within tumours also offer malignant cells a port of entry into the circulation. Ultrastructural studies indicate that the endothelium of tumour-associated vessels is often defective and such vessels may be particularly susceptible to penetration by tumour cells[13]. Zones of reduced oxygenation[14] and overt necrosis[3,9,12] are common in tumours and the blood vessels in such regions show structural alterations (defects) that could facilitate invasion by tumour cells.

Some of the clinical literature still pays homage to the view that carcinomas tend to spread via lymphatics, whereas malignant tumours of mesenchymal origin spread via the blood system. There is now substantial evidence that malignant cells can pass freely between lymphatics and blood vessels[2,5]. The belief that particular tumours spread exclusively via one or other pathways is a misleading oversimplification, which does not offer a reliable basis for predicting either the distribution of metastases or the choice of therapy[4].

Following penetration of blood vessels, tumour cells are either carried away passively in the bloodstream or remain at the site of vessel invasion where they proliferate and continue to shed emboli into the circulation[2]. Shedding of emboli is exacerbated by intermittent changes in venous pressure, by turbulent alterations in blood flow and by movements or manipulation of the neoplasm during diagnostic tests or surgery[15].

The distribution of metastases

The presence of tumour cells in the circulation is necessary, but certainly not sufficient, to produce metastasis[2–5,15]. As discussed later, most tumour cells that enter the circulation do not survive to complete the process.

Knowledge of the expected tissue distribution of metastases arising from primary tumours in different anatomical locations is important to the clinician who must monitor the progress of a cancer patient and plan appropriate therapy. In man, this information has come from descriptive observation of the distribution of metastases arising from particular types of tumours. More accurate information on the behaviour of circulating tumour cells has been obtained in experimental animals by studying the distribution and fate of radiolabelled tumour cells[2].

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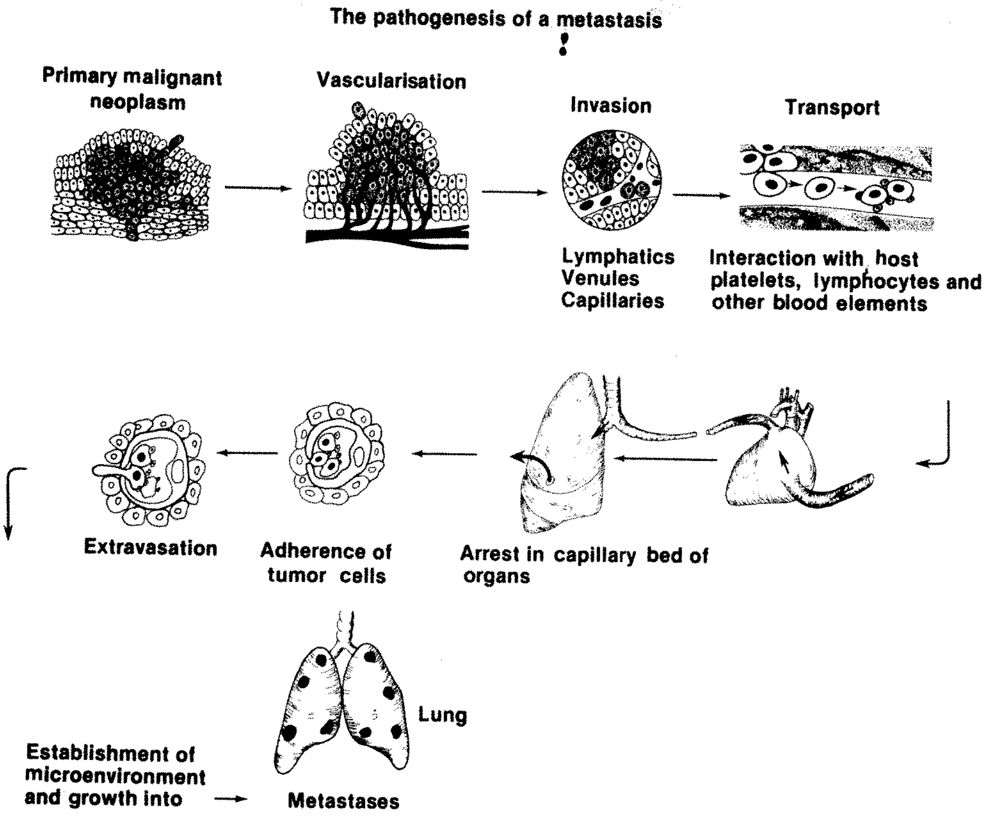
The pathogenesis of a metastasis
Primary malignant neoplasm
Vascularisation
Invasion
Transport
Lymphatics
Venules
Capillaries
Interaction with host platelets, lymphocytes and other blood elements
Extravasation
Adherence of tumor cells
Arrest in capillary bed of organs
Lung
Establishment of microenvironment and growth into
Metastases

Fig. 1 Schematic illustration of the major steps in the pathogenesis of metastasis. Initial neoplastic transformation of susceptible cells gives rise to a small population of tumour cells. Vascularisation of this early neoplastic lesion allows further proliferation of tumour cells and enlargement of the primary tumour. Malignant cells within the primary tumour next begin to invade the surrounding host tissue(s). Entry of invading tumour cells into lymphatics or blood vessels serves to transport them to distant sites in the body where they lodge and arrest in the capillary beds of various organs. For illustrative purposes this figure depicts arrest of malignant cells in the lung capillary bed. The arrested cells then exit from capillaries into the surrounding lung parenchyma where, subject to provision of a suitable environment, they proliferate to form metastases. A more detailed discussion of each of these steps is given in the text. (Figure reproduced with permission from ref. 103).

Some degree of nonspecific trapping and arrest of circulating tumour cells in the microcirculation occurs simply as a result of mechanical factors. The size and deformability of tumour cells, the diameter and distensibility of capillaries and the interaction of tumour cells with each other and with circulating host cells to create multicellular emboli are all relevant in this context[2-4,10].

Although mechanical factors undoubtedly exert some effect on the distribution and viability of circulating tumour cells, it is now clear that mechanical factors alone are not sufficient to explain the site(s) of tumour cell arrest and metastasis formation in all human and animal tumours. Clinical observations in man suggest that certain tumours consistently metastasise to particular organs[2,12,16-18]. Non-random patterns of tumour cell arrest and subsequent metastasis formation have also been described in a variety of animal tumour systems[19-26]. Also, experimental studies in which radiolabelled tumour cells are introduced into the circulation have shown that in many instances circulating cells arrest in a wide variety of organs but metastases occur only in certain organs[2,3]. Why some organs support metastatic growth, while others that receive viable tumour cells do not, remains unknown.

Recent evidence indicates that the properties of circulating tumour cells can affect their arrest pattern. A remarkable demonstration of the ability of tumour cells to arrest and grow preferentially in particular organs is provided by experiments showing that animal tumour cells which ordinarily arrest in the lung also localise and grow in pieces of lung implanted subcutaneously (refs 24, 27 and I. R. Hart, unpublished data). In contrast, tumour growth does not occur in subcutaneous implants of other organs. Comparable findings have been obtained in experiments in which the melanoma cells shed from pulmonary metastases in one animal were found to localise preferentially in the lung of non-tumour bearing parabiotic animals[28].

Equally dramatic evidence of the influence of tumour cell properties on the arrest pattern of circulating cells has come from recent work on the experimental manipulation of plasma membrane composition in B16 melanoma sublines of differing metastatic potential[29]. In these experiments, plasma membrane components were transferred between different sublines by taking advantage of the fact that B16 melanoma cells growing *in vitro* and *in vivo* spontaneously shed closed vesicles of intact plasma membrane which can be collected and fused with other cells using polyethylene glycol. Membrane vesicles from the B16–F10 subline, which has a high capacity for lung colonisation, were fused with B16–F1 cells, which produce few pulmonary metastases. When the vesicle-modified cells were injected intravenously into C57BL/6 mice they were found to arrest in the lung capillary bed in significantly greater numbers in the lung capillary bed than untreated B16–F1 cells (Fig. 2). Equally important, increased cell arrest resulted in increased production of pulmonary metastases[29] (Table 1). The possibility that the increased arrest of vesicle-modified B16–F1 cells in the lung was due simply to increased cell volume produced by vesicle uptake was excluded by showing that the volume of vesicle-treated cells (determined from cells isolated by centrifugal elutriation) did not differ significantly from control B16–F1 cells[29].

In related experiments, lymphocyte-resistant sublines of B16–F1 were rendered susceptible to *in vitro* killing by cytotoxic lymphocytes by treatment with vesicles from lymphocyte-sensitive B16 sublines plus polyethylene glycol[29]. Since lymphocyte-mediated cytolysis occurs only when the target antigen resides within the plasma membrane[30-32], this experiment also shows that the vesicles fuse with the cellular plasma membrane and are not merely adsorbed to the cell surface.

The effect of B16–F10 vesicles in modifying the arrest of B16–F1 cells was highly specific. Vesicles from B16–F10$^{\text{lr}}$ cells (a variant of the B16–F10 subline that is resistant to killing by syngeneic immune lymphocytes, but which resembles B16–F1 in its arrest and metastatic behaviour) had no effect on the arrest of B16–F1 cells (Fig. 2). Interestingly, fusion of B16–F1 vesicles with B16–F10 cells did not alter the arrest or metastatic behaviour of these cells (Fig. 2) but this may have been because the vesicles were not introduced in sufficient amounts.

Studies using B16–F1 vesicles surface-labelled with ^{125}I or ferritin revealed that vesicle components persist in the plasma membrane of B16–F10 acceptor cells for only 18–24 hours[33].

How then does vesicle treatment enhance metastasis formation when proliferation of extravasated cells to form metastases does not begin until 1–2 days after injection[3,29] when vesicle components are no longer present? Other studies have shown that B16 cells invade the blood vessel wall within 2–4 hours after their arrest[33]. Once within the vessel wall, the tumour cells are less susceptible to damage by haemodynamic forces or circulating host cells and therefore have a greater opportunity of continuing the process of metastasis. Thus, by simply increasing the efficiency of cell arrest and invasion in the lung capillary bed, vesicle-induced modification of B16–F1 cells would increase the likelihood of metastasis formation even though vesicle components *per se* will make no direct contribution to any events occurring 24 hours after initial cell arrest.

Ultrastructural studies indicate that tumour cells adhere to the endothelium and also to the underlying basement membrane[2,13]. Recent studies by one of us (G.P.) suggest that it may be profitable to concentrate further studies on the subendothelial basement membrane of blood vessels since it seems to offer a better substrate for adhesion of malignant tumour cells than does the endothelium (Table 2). Also, tumour cells from clones of a murine fibrosarcoma with high metastatic potential adhere to the endothelial basement membrane more efficiently than clones of low metastatic potential (Table 2).

Following arrest and implantation in the vessel wall, tumour cells must exit from the vessel into the surrounding tissue(s). Extravasation of malignant cells is believed to involve mechanisms similar to those responsible for the initial invasion of blood vessels. Recent observations showing that spontaneously metastatic B16 melanoma sublines produce high levels of type IV collagenase[34] may be particularly significant in this context since type IV collagen is the predominant form of collagen in the basement membrane of capillaries[34].

Successful extravasation and limited proliferation of malignant cells creates small colonies of tumour cells, so called micrometastases, which at first surround blood vessels[35]. Further growth of these lesions to form macroscopic metastases of clinical importance requires ingrowth of new blood vessels to provide adequate nutrition to support cell proliferation. The ability of certain tumour cells to release a factor(s) that stimulates growth of new blood vessels (angiogenesis)[35] may be of advantage in this regard. It is important to note, however, that angiogenic factors are also produced by host lymphocytes and macrophages[35]. Release of angiogenic factors from these host cells as a consequence of interaction with metastatic cells, together with release of cell growth stimulating factors by host platelets, could create a situation in which the normal host homeostatic mechanisms increase the risk of metastasis.

Table 1 Formation of experimental metastases by B16 melanoma sublines and B16 sublines treated with plasma membrane vesicles

Cell type	Plasma membrane vesicles	PEG/PHA†	Average number of pulmonary metastases*
B16-F1	None	−	16±7
B16-F1	None	+	14±6
B16-F10	None	−	114±24
B16-F10	None	+	87±16
B16-F1	B16-F10	−	19±8
B16-F1	B16-F10	+	44±11
B16-F10	B16-F1	−	103±16
B16-F10	B16-F1	+	94±19
B16-F1	B16-F1	+	11±6
B16-F10	B16-F10	+	91±15

The B16 mouse melanoma cell sublines were incubated with purified preparations of plasma membrane vesicles from the indicated donor cells in the presence (+) or absence (−) of PEG and PHA for $\frac{1}{2}$ h at 37 °C as described in Fig. 2. Control cell populations which were not treated with plasma membrane vesicles were incubated in serum-free Dulbecco's modified Eagle's medium for $\frac{1}{2}$ h at 37 °C. Vesicle-treated and control cell populations were then tested for their ability to form experimental metastases after i.v. injection into 10-week-old C57BL/6 mice (5×10^4 cells per animal) as described in the legend to Fig. 2. Formation of experimental metastases was measured 18 days after injection of cells.

† PEG = polyethylene glycol; PHA = phytohaemagglutinin.

* Mean values (±s.d.) derived from measurements on 20 mice per group in two separate experiments. (Table derived from data published in ref. 29).

The immune response and metastasis

Throughout the metastatic process, malignant cells are subject to attack by host defence systems, which can be immune[36,37] or nonimmune[38] in nature. Despite an enormous effort to define the role of immune mechanisms in the recognition and destruction of tumour cells, their contribution remains unclear, and convincing evidence that they influence the outcome of neoplastic disease in man is still lacking.

The classic 'immune surveillance' theory[39] attributes host resistance to tumours to the destruction of susceptible tumour cells by mature immune T lymphocytes. This hypothesis, although widely accepted at first, has come under increasing criticism in recent years. The following findings have been cited as evidence against a central role for host immunity in surveillance against neoplastic cells: the incidence of spontaneous tumours in T cell-deficient nude mice is no higher than in immunocompetent animals; most tumours that metastasise in normal immunocompetent animals fail to do so in nude mice; some murine melanoma cells that are resistant to killing by T lymphocytes are less metastatic than cells that are susceptible to killing by T cells; and tumour growth rate in immunologically privileged sites is no faster, and often slower, than that of neoplasms in immunologically exposed sites (see refs 2, 40–44). Also, spontaneous tumours in both man and animals frequently lack detectable antigenic alterations[45,46] and might thus avoid recognition by the immune system. This conclusion comes, however, from experiments designed to detect transplantation resistance and other immune responses involving T cells. The possibility that tumour cells express antigenic or other surface changes recognised by other host defence cells such as natural killer (NK) cells or macrophages still has to be tested (see below).

Experimental studies on the role of host immunity in cancer metastasis have yielded contradictory results. In many transplantable tumour systems, depression of host immunity increases the incidence of experimental and spontaneous metastasis (see ref. 47); in other tumour systems, depression of immunologic reactivity decreases, or even prevents, metastasis[48–51] or has no influence on the growth of a local or disseminated tumour[52]. The basis for these discrepancies could be that the studies were carried out with tumours of various histologic types, of different aetiologies, and induced in different species. We have attempted to minimise these variables by testing three C3H fibrosarcomas with different degrees of immunogenicity for their metastatic activity in immunosuppressed normal, sham-suppressed and immunologically reconstituted syngeneic mice[52]. The highly immunogenic fibrosarcoma formed more pulmonary tumour colonies in immunosuppressed mice than in normal, sham-suppressed (sham-thymectomy and 450R) or reconstituted animals (thymectomy, X-ray irradiation, plus 10^7 normal syngeneic lymphocytes intravenously). A fibrosarcoma of intermediate immunogenicity also formed more pulmonary metastases in immunosuppressed recipients, but this increase could not be reversed by reconstitution with 10^7 normal lymphocytes. In contrast, the least immunogenic tumour formed fewer pulmonary tumour colonies in immunosuppressed mice than in normal, sham-suppressed or reconstituted mice. Thus, even in these relatively uniform conditions, the influence of the immune system on experimental cancer metastasis varied for different tumours. These data also show that tumour immunogenicity is an important factor in the relationship between host immunity and tumour dissemination[52].

Table 2 Adhesion of non-neoplastic cells and metastatic tumour cell variants to endothelial cells and subendothelial basement membrane

Cell	Metastatic potential*	% Cell adhesion‡ Intact endothelium	Subendothelial basement membrane
C57BL/6 mouse embryo cells	None	35.6±1.4	30.2±1.2
B16-F1 melanoma	Low	39.2±1.7	46.4±1.4
B16-F10 melanoma	High	33.4±0.9	69.7±1.5
B16-F1-BV8 melanoma†	High	36.1±1.3	83.7±2.3
C3H mouse embryo cells	None	35.2±0.9	31.6±1.1
UV-2237 fibrosarcoma, clone 15	Low	27.4±1.1	39.3±1.3
UV-2237 fibrosarcoma, clone 46	Low	38.7±1.3	49.2±1.6
UV-2237 fibrosarcoma, clone 38	Low	31.9±1.5	48.6±1.5
UV-2237 fibrosarcoma, clone 34	Medium	39.4±0.9	64.2±1.6
UV-2237 fibrosarcoma, clone 9	Medium	30.7±1.2	67.5±2.0
UV-2237 fibrosarcoma, clone 39	High	45.2±1.8	65.8±1.7
UV-2237 fibrosarcoma, clone 25	High	37.4±1.3	80.4±1.5

Actively growing monolayer cultures of the indicated cell types were labelled with ^{125}I-IUdR as described in the legend to Fig. 2. Aliquots containing 2.5×10^4 radiolabelled cells were then plated onto the surface of 1 cm^2 segments of human umbilical vein in 0.1 ml Dulbecco's modified Eagle's medium containing 10% calf serum. The radioactivity present in five representative inocula of each cell type was measured in a Packard gamma counter. After addition of radiolabelled cells the blood vessel segments were incubated for 1 h at 37 °C. The culture medium plus non-attached cells was then aspirated and the surface of the blood vessel was washed twice with prewarmed Hanks' balanced saline solution (HBSS). The amount of radioactivity associated with the blood vessel segments was then measured and expressed as a percentage of the total radioactivity in the original inoculum. In assaying cell adhesion to intact endothelium the structural integrity of the endothelium was confirmed by scanning electron microscopy. Vessels showing partial detachment of endothelial cells or regions of complete loss of endothelial cells were excluded from the adhesion assay. For assays of cell adhesion to the subendothelial basement membrane, a balloon catheter was used to remove the majority of endothelial cells after which the vessel was treated with 0.5% EDTA to remove any remaining endothelial cells. Complete removal of endothelial cells was then confirmed by scanning electronmicroscopy.

* Ability to form experimental pulmonary metastases after i.v. injection of 1×10^5 cells. The classification refers to median number of experimental metastases produced in 10 animals[70,71]: low (1–15); medium (30–50) and high (>100).

† A variant isolated from the B16-F1 subline by *in vitro* selection for enhanced ability to invade the wall of blood vessels maintained *in vitro* in a perfusion culture system[78].

‡ Mean±s.e.m.

The existence of a system of natural host defence against tumour cells mediated by natural killer (NK) cells[42] offers a possible alternative mechanism for cell-mediated surveillance against circulating neoplastic cells. The role of NK cells, and also activated or armed macrophages[53], in limiting tumour growth and metastatic spread, as well as the related questions of amplifying NK- and macrophage-mediated destruction of tumour cells[54,55] as methods for treating micrometastases are presently being studied in many laboratories and the outcome of this work is awaited with considerable interest.

Metastasis as a selection process and variation in the metastatic capabilities of tumour cells

Metastasis is a highly selective process. The malignant cells that complete all of the steps described in the previous section and establish metastases have survived a demanding sequence of hostile events which destroy the majority of tumour cells[2–4]. For example, only a small proportion of the total cells from the primary tumour may gain access to the circulation and of these as few as 0.1% may survive the trauma of dissemination and arrest[2]. Surviving cells suffer further loss during extravasation and the viability of extravasated cells, in turn, may depend on the availability of appropriate nutrients and escape from destruction by host defence systems.

Recognition that only very few tumour cells survive to develop into metastases raises the important question of whether survival is random or non-random. Random survival would mean that the primary tumour is populated by cells of equal metastatic potential, any one of which could give rise to a metastasis. The alternative possibility is that metastasis is not random, but involves the survival of specialised tumour cells endowed with specific properties that befit them to complete each step in the metastatic process. In this case, the primary tumour would contain subpopulations of cells of differing metastatic potential, including cells with little or no capacity to metastasise.

The possibility that cells with high metastatic potential can be isolated from a heterogeneous parental tumour by selection procedures was first suggested in 1939 by Koch who isolated a highly metastatic subline from the Ehrlich carcinoma tumour by serially transplanting lymph node metastases[56]. In 1955, Eva Klein demonstrated that gradual conversion of some solid murine neoplasms into ascites variants was due to the selective overgrowth of a small number of cells which differed from the parental population in their ability to proliferate in the peritoneal cavity and metastasise to the lungs[57]. Since the change was stable and heritable, Klein concluded that the gradual conversion of the solid tumour to the ascites form involved mutation/selection and not adaptation. Further evidence of heterogeneity in the metastatic potential of tumour cells has come from more recent experiments in which selective harvesting of cells from metastases during successive passages of tumour cells *in vivo* has yielded cell populations with a greater metastatic potential than cells from the original cell population[19,22,58–70]. In this type of *in vivo* selection the isolated cell populations are not cloned, and the enhanced metastatic potential detected in the population as a whole is due to enrichment of the proportion of highly metastatic cells in the population at each successive passage.

Definitive evidence that malignant primary tumours contain subpopulations of cells with differing metastatic capabilities was obtained in 1977 by Fidler and Kripke[70] using the B16 melanoma syngeneic to the C57BL/6 mouse. To investigate whether primary tumours contained cells of differing, or uniform, metastatic potential they prepared a cell suspension from a subcutaneous primary tumour and divided it into two aliquots. One part was immediately assayed for its ability to form experimental pulmonary metastases following intravenous injection (a procedure for assaying cellular metastatic ability which has been validated elsewhere[71]). From the second part of the original suspension, 17 clones were isolated and their progeny tested for their ability to produce experimental metastases. If the tumour was populated by cells of uniform metastatic potential, then the cloned sublines should each produce the same number of metastases as the uncloned parental population. This was not the case. The original uncloned parental tumour cell population produced similar numbers of metastases in different animals but the cloned sublines differed markedly in their metastatic potential (Fig. 3). Although the number of metastases produced by any given clone was constant, individual clones showed wide variation in their metastatic potential. Control subcloning experiments demonstrated that the cloning process was not responsible for the variability[70]. These experiments thus suggest that the parent B16 tumour is heterogeneous and that highly metastatic tumour cell variants preexist in the parental population[70].

To exclude the possibility that the metastatic heterogeneity found in B16 melanoma clones might have been introduced as a

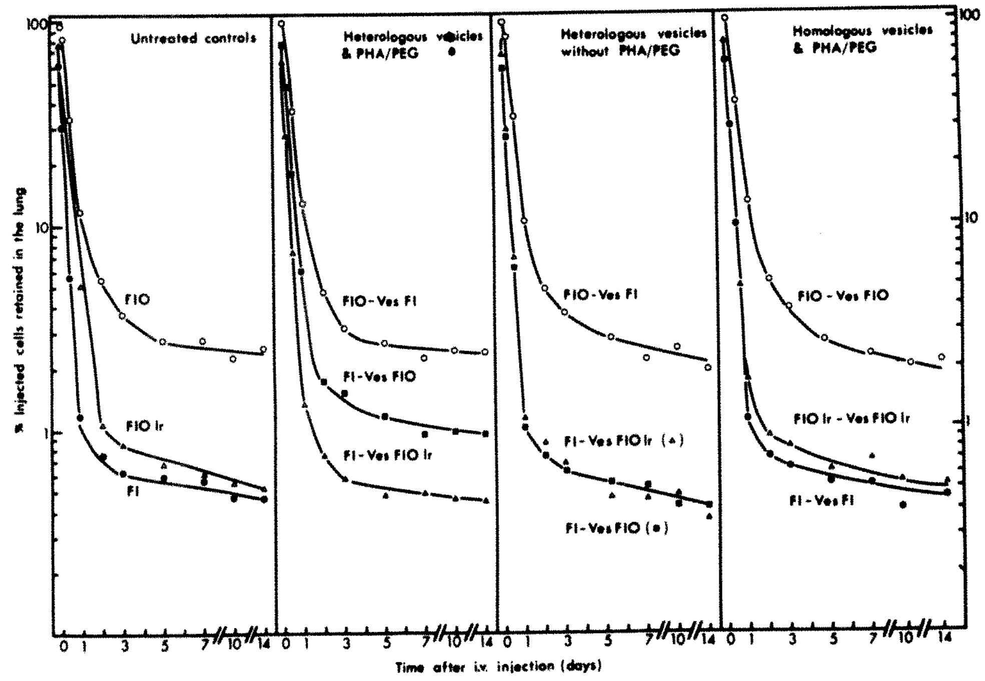

Fig. 2 Lung arrest and retention of ^{125}I-IUdR labelled B16 melanoma sublines and B16 sublines treated with plasma membrane vesicles after i.v. injection into C57BL/6 mice. Plasma membrane vesicles (Ves-F1; Ves-F10$^{\mathrm{lr}}$; Ves-F10) were purified from the culture medium of actively growing roller bottle cultures of the various B16 sublines and were purified as described elsewhere[29]. ^{125}I-IUdR-prelabelled cells from the indicated B16 sublines (F1, low lung metastasis; F10, high lung metastasis, F10$^{\mathrm{lr}}$, lymphocyte-resistant variant of F10 with low metastatic capacity) were incubated in suspension with homologous or heterologous vesicles (300–400 μg protein per 10^7 cells) in serum-free Eagles minimum essential medium (MEM) with or without 50 μg ml^{-1} PHA-P and PEG 6000 as described elsewhere[29]. Control cells were incubated without vesicles in serum-free MEM for the same period. Vesicle-treated and control cells were then washed three times with prewarmed Hanks' balanced saline solution (HBSS) and incubated for 10 min at 37 °C in 0.2% EDTA to separate multicellular clumps into single cells. Since cell size can influence cell arrest in the microcirculation, vesicle-treated and control cells injected into animals (see below) were subjected to density gradient centrifugation and centrifugal elutriation to obtain cell fractions of similar size and density[29]. Aliquots of 1×10^5 viable, radiolabelled cells with a modal volume in the range $1.4–1.6 \times 10^3$ μm^3 were then injected as a single cell suspension in 0.2 ml HBSS into the tail vein of unanaesthetised C57BL/6 mice. The radioactivity present in five representative inocula was monitored for each cell preparation in a gamma counter. Lungs were collected from injected mice at intervals from 5 min to 14 days after injection and placed in vials containing 70% ethanol. The ethanol was changed daily for 3 days to remove alcohol-soluble radioactivity. The remaining lung-associated radioactivity represents radioactivity incorporated into the DNA of tumour cells that were viable at the time the animals were killed[104]. Lung-associated radioactivity at the indicated times after injection is expressed as a percentage of the injected cells retained in the lung. Each point represents a mean value derived from measurements of five separate animals. The arrest kinetics of ^{125}I-IUdR-labelled F1, F10 and F10$^{\mathrm{lr}}$ cells treated with PHA/PEG but not exposed to vesicles did not differ from that shown in the left-hand panel for untreated control cells from these sublines. Figure constructed from data given in ref. 29.

result of the lengthy *in vivo* and *in vitro* cultivation that this tumour has undergone since its isolation in 1954, Kripke and Fidler repeated the same experiment with a newly induced primary tumour and tested the metastatic potential of cells isolated from this tumour after only 6 passages *in vitro*. The results obtained with this tumour, a UV-induced fibrosarcoma (UV-2237) of a C3H mouse, were similar to those obtained with the B16 melanoma. Similarly, marked heterogeneity in metastatic potential has now been demonstrated in cloned populations isolated from a murine mammary tumour[72], a murine MCA-induced fibrosarcoma[73], a murine sarcoma virus-transformed fibrosarcoma[26] and a transformed rat liver epitheloid cell line[74]. More recently, we have shown that whereas clones isolated from the primary tumour show widely differing metastatic abilities, cloned sublines isolated from individual metastases exhibit a much more uniform metastatic potential. This suggests that metastases, in contrast to the primary tumour, are more homogeneous and populated by cells with similar metastatic capabilities (Fig. 3).

Metastatic and non-metastatic tumour cell variants derived from the same parent tumour cell population have also been isolated using *in vitro* selection methods in which cell populations are selected for expression (or failure to express), a property considered of possible importance in one or more steps in the metastatic process. The metastatic behaviour of such isolated cells is then assayed *in vivo* to determine whether the particular selection has enriched the population for cells with increased or decreased metastatic capacity. Selection *in vitro* for detachment from a monolayer[75], resistance to lysis by lymphocytes[76], resistance to lectin-mediated toxicity[77], attachment to collagen[58] and ability to invade various tissues maintained in organ culture[78] have now been used successfully to isolate tumour cell lines with increased or decreased metastatic capabilities.

Collectively, this large body of evidence indicates that malignant tumours are not uniform entities populated by cells of equal metastatic potential. Rather, primary tumours are populated by subpopulations of cells of widely differing metastatic potential. This conclusion supports the proposal made by Nowell[79] that during tumour progression variant cells arise which are subject to host selective pressures that favour the survival of variants with increased malignant capacity. Regardless of whether neoplasms are monoclonal or multiclonal in origin[79,80], by the time they can be diagnosed clinically they could be heterogeneous and contain subpopulations of cells with a wide range of phenotypic characteristics. Recent studies by one of us (I.J.F.) using mouse fibroblasts transformed *in vitro* by cloned stocks of murine sarcoma virus have shown that extensive heterogeneity in metastatic potential is present in subclones derived from the original transformed cells as little as 28 days after the initial transformation.

Heterogeneity in the metastatic potential of cells in the primary tumour is perhaps not unexpected. Cells populating both human and animal neoplasms are known to be heterogeneous with respect to their immunogenicity[83], antigenic properties[82–84], growth rate[85], metabolic characteristics[86], hormone receptors[87], pigment production[88], radiosensitivity[89] and susceptibility to cytotoxic drugs[90–92]. Of even more interest in the context of the present discussion are recent observations showing that tumour cells in primary and metastatic lesions differ in their antigenic properties[93], biochemical characteristics[94] and response to cytotoxic drugs[61,95–97].

Implications

The presence within the primary tumour of subpopulations of cells with differing metastatic capabilities has profound implications for the way in which research is done to identify the cellular properties responsible for metastasis. Hitherto, the general experimental approach to this problem has been to study cell populations isolated at random from the primary tumour and to assume that properties detected in these cells are also expressed by cells that give rise to metastases. The demonstration of metastatic heterogeneity in the cells of the primary tumour indicates that this assumption is not valid. Experimental efforts to identify features unique to metastatic subpopulations will require isolation of such subpopulations and comparison with nonmetastatic (but tumorigenic) subpopulations of cells from the same parent tumour. Continued efforts to study the properties of malignant cells using unselected heterogeneous cell populations will probably be of little value since the proportion of nonmetastatic or poorly metastatic cells may be sufficiently high to mask properties peculiar to small subpopulation(s) of highly metastatic cells. Moreover, studies that utilise tumour cells isolated from tumours whose detailed origins are not known or tumours propagated by serial transfer intraperitoneally may be unproductive in that the former may not be syngeneic to the recipient animals while the latter may represent a selected subpopulation of cells since growth intraperitoneally has been shown to be selective[57].

The existence of specialised subpopulations of cells with high metastatic potential has equally far reaching consequences for the testing of potential agents for treating metastatic disease. Both human and animal neoplasms contain subpopulations of cells with differing sensitivities to cytotoxic drugs[90–92]. Differences in the response of primary and metastatic lesions to therapeutic agents is well documented in clinical practice[98] and metastatic cells showing increased resistance to chemotherapeutic agents have been selected in experimental animal tumours[61]. As pointed out recently by Schabel *et al.*[98], most of the methods presently used to define the drug sensitivity of experimental animal tumours might be misleading since they are based on the belief that transplantable tumours are homogeneous, that is, uniform entities. Assays of the antitumour activities of therapeutic agents that rely on partial regression of the primary tumour may be inadequate for screening agents for

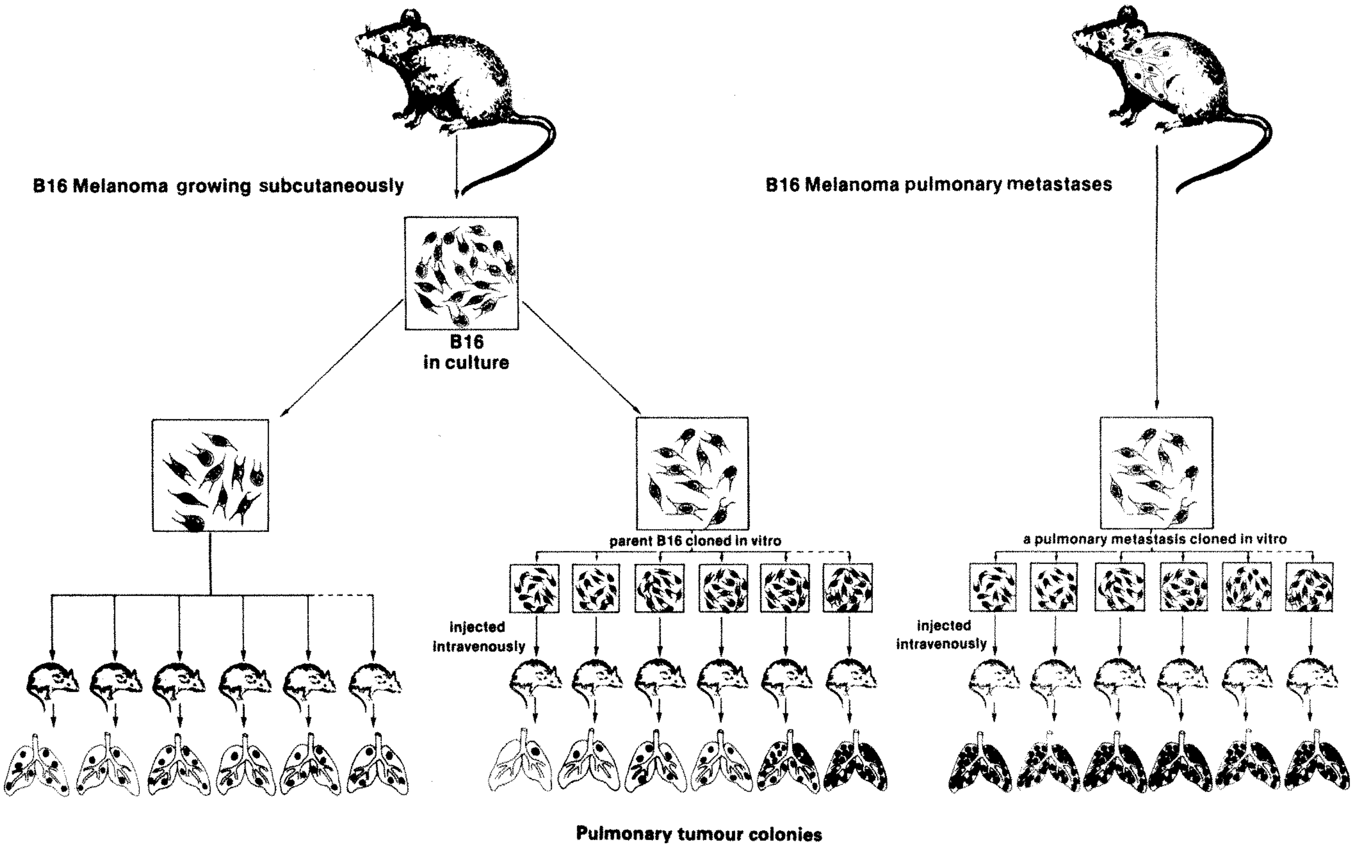
B16 Melanoma growing subcutaneously
B16 in culture
B16 Melanoma pulmonary metastases
parent B16 cloned in vitro
a pulmonary metastasis cloned in vitro
injected intravenously
injected intravenously
Pulmonary tumour colonies

Fig. 3 Schematic illustration of experiments demonstrating heterogeneity in the metastatic capabilities of clones derived from cells in a parental murine tumour[70] and the relative homogeneity of the metastatic potential in clones derived from cells isolated from a pulmonary metastasis (G.P. and I.J.F., unpublished data). The experiment was similar in design to the classical fluctuation test devised by Luria and Delbruck to distinguish between selection and adaptation in the origin of bacterial mutants[105]. Cells isolated from the parental tumour were divided into two parts. One part (left-hand side of figure) was injected i.v. into 60 C57BL/6 mice to assay formation of experimental pulmonary metastases. The other part (central portion of the figure) was used to produce a series of clones and the progeny of each clone was injected i.v. (10 mice per clone) in an identical procedure to that of the uncloned parental tumour cells. Mice were injected i.v. with 5×10^4 viable cells and the number of lung metastases was measured 18 days later by examining the lungs with a dissecting microscope[70]. Cells of the parental population produced a median of 40 lung metastases (range 8–131 metastases). In contrast, cells of clones from the parental tumour differed markedly in their metastatic capabilities, from very low (range of median number of metastases from 7 clones = 3–20), medium (medians from 3 clones = 36–99 metastases), high (medians from 5 clones = 150–260 metastases) to very high metastatic capacity (medians from 2 clones = >500 metastases)[70]. In contrast, clones derived from cells isolated from a single pulmonary metastasis (right-hand side of figure) were of uniform metastatic potential when assayed in similar experimental conditions. This indicates that the metastatic lesion is composed of a more homogeneous cell population with a relatively uniform metastatic capability. The clones shown in the figure produced a uniformly high number of metastases. Although the number of metastases produced by cells isolated from any given metastasis is uniform, clones isolated from different metastases may differ substantially in the number of metastases they produce (G. P. and J. Doll; I. J. F., E. Gruys and M. L. Kripke, unpublished data). This reflects differences in the metastatic activities of the original tumour cell subpopulations residing within the parental tumour which give rise to individual metastases[70].

their antimetastatic activity. Assays in which a drug, or combination of drugs, are found to limit growth of the primary tumour to 50% of that in untreated controls may merely reveal the presence of a subpopulation(s) of drug-sensitive cells and do not necessarily offer any insight into the pharmacologic susceptibility of the tumour cell subpopulations that will give rise to metastases. Future efforts to identify potential agents for treatment of metastases might thus more profitably adopt assays in which therapeutic agents are screened for their activity against cloned tumour cell subpopulations with defined metastatic capabilities.

Phenotypic heterogeneity is not only a problem for the chemotherapist. Immunologic heterogeneity among tumour cells[82–84, 93, 99] poses equally serious problems for efforts to treat neoplastic disease by specific immunotherapy[100]. For example, analysis of a number of AKR mouse lymphomas of recent origin has shown that these tumours are immunologically polyclonal, consisting of a numerically dominant subpopulation and several distinct minor subpopulations that comprise less than 3% of the tumour. Attempts to induce tumour rejection by immunising tumour-bearing animals using unfractionated thymoma cells were unsuccessful since effective immunisation occurred only against the dominant clone and the minor subclones did not offer a sufficient immunologic mass to stimulate the immune response[101]. Immune rejection of the dominant clone merely permits the minor subpopulations to proliferate and become dominant. This situation is analogous to the well documented problem of drug-resistance in cancer chemotherapy where resistant variants preexist within the parental tumour population, albeit as a minor subpopulation, but following destruction of the original dominant drug-sensitive population(s) they proliferate unchecked[102].

Recognition that primary malignant tumours are not populated by cells of equal metastatic potential, but contain subpopulations of cells with widely differing metastatic capabilities, calls for critical reappraisal of the adequacy of many of the experimental systems presently used in studying 'malignancy' and in screening agents for treating metastatic disease. Experimental systems that fail to take account of the heterogeneity of metastatic potential of neoplasms may prove to be less successful in providing insight into the basic mechanisms involved in metastasis than systems that embrace this concept. We thus consider the isolation and characterisation of tumour cell variants of defined metastatic potential from human and animal neoplasms to be of major importance for future progress in metastasis research.

The search for a property uniform to all metastatic cells may be unproductive. Work carried out with isolated variant tumour cell lines must consider that the development of metastases is dependent on an interplay between host factors and the intrinsic properties of the tumour cells. To establish metastases, tumour cells must complete *all* steps involved in the metastatic process. Enhanced performance of a tumour cell in one step of the process cannot compensate for an inability to complete a subsequent step. The failure of most tumour cells to produce metastases need not be due to a single, common factor. Interruption of the metastatic sequence at any step in the process will prevent the production of clinical metastasis. On a more optimistic note, the increasing availability of tumour cell populations with differing metastatic capabilities should facilitate experimental efforts to identify the properties which enable malignant cells to metastasise. With the availability of this knowledge it might then be possible to design more effective procedures for the treatment of this catastrophic disease.

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ARTICLES

New results from optical polarimetry of Saturn's rings

Paul E. Johnson, James C. Kemp, Randall King, Timothy E. Parker & Mark S. Barbour

Department of Physics, University of Oregon, Eugene, Oregon 97403

Linear polarimetry of Saturn's rings, obtained through the period of the 1979 opposition, is presented. The polarisation clearly correlates in direction with the plane containing the Sun, planet and Earth, but not the ring plane. The results are consistent with local scattering on the surface of individual ring bodies, covered with frost.

SATURN'S rings have intriguing and rather special optical properties. The rings undergo a sharp surge in brightness during a narrow phase interval around opposition[1], when the Sun, Earth, and Saturn are aligned. Water ice in some form is present on the rings and apparently controls their optical behaviour[2,3]. The geometrical arrangement of the ring particles—either a single monolayer of bodies or a thicker (low-density) disk—has long been a subject of speculation[4]. The typical size of a ring body is poorly known, with diameter estimates ranging from 1 cm to 1 km (ref. 5). One Earth-based tool which is useful for probing the nature of the rings is the measurement of the polarisation of light reflected from the rings.

Polarisation theory

The laboratory investigations of Lyot[6], Dollfus[6,7], and others have provided much information on the polarisation of light reflected from terrestrial and lunar materials. These studies have shown that the variation of polarisation with phase angle (angle between the incident and emergent light rays) is diagnostic of both the bulk properties and the texture of the surface material.

The polarisation of light reflected from a flat, polished surface is polarised so that the electric vector (**E** vector) of the polarised component is parallel to that surface, in compliance with Fresnel's laws of reflection. Typical Solar System bodies, however, tend to be covered by either a particulate or rough and pitted surface, presenting an ill-defined surface boundary. The light scattered from such a surface is found to be polarised with the electric vector lying either in the scattering plane (termed negative polarisation) or perpendicular to that plane (positive polarisation), depending on the phase angle and other parameters. The so-called negative branch is confined to a narrow interval around zero phase, that is, around opposition.

On the grounds of general symmetry, we might expect two types of contributions to the polarisation of Saturn's rings, one correlated with the ring plane, the other with the scattering plane (the plane including the Sun, planet and Earth). The idea of a ring-plane polarisation is particularly attractive because Saturn's rings are extremely thin and flat within telescopic resolvability; and physical mechanisms can easily be cited for such an effect. One characteristic of this type of polarisation, correlated in direction (position angle) with the ring plane, is that it should not vanish at precise opposition. It would be seen most clearly at opposition, as other polarisation effects correlated only with the scattering plane would then vanish, the scattering plane being undefined at zero phase. We will show that the absence of correlation with the ring plane means that any polarising mechanism related to the ring plane, as such, must be ineffective.

Only a few investigations of the linear polarisation of the light reflected by Saturn's rings have been published since Lyot's original discovery in 1923 [6–10]. Lyot[6] attempted to explain the polarisation of the rings as being due to two or three independent mechanisms: (1) direct scattering of sunlight by individual ring bodies; (2) scattering of light from Saturn's disk, by the rings; and (3) multiple scattering between aligned ring bodies. Mechanisms (2) and (3) would produce a polarisation of the type with a position angle related to the ring plane. Dollfus[9] finds a component in the polarisation of the B ring with a position angle directed either radially or tangentially, relative to the centre of Saturn, unexpectedly varying in time (on a time scale as short as several days). He reports that this rapidly variable component can be as large as 0.2%. Our measurements, with those in ref. 8, show no evidence for such a component. Rather, we find that polarisation varies smoothly with phase angle.

Observations

Figures 1 and 2 show the linear polarimetry of Saturn's rings obtained between December 1978 and May 1979. The data consist of two independent subsets, acquired at different observatories, each with a separate polarimeter and observing team. Measurements at the Pine Mountain Observatory of the University of Oregon (PMO) were made (by J.C.K., T.E.P. and M.S.B.) using the 61-cm telescope. A 41-cm telescope was used at the Kitt Peak National Observatory (KPNO) (by P.E.J. and R.K.). All PMO observations used a 7 arc s aperture, while at KPNO an 11 arc s aperture was used. Measurements were made with either a standard B filter or a yellow interference filter (V') having $\lambda_{\text{eff}} = 5{,}530$ Å and $\Delta\lambda = 700$ Å.

Both photoelastic-modulator polarimeters have been checked (in connection with other work) against both polarised and unpolarised standard stars; each has a typical[11] measured instrumental polarisation $\leq 0.01\%$. Excellent agreement between the two subsets of data (better than $\pm 0.02\%$ in ΔP for the B-filter data, and $\pm 2°$ in $\Delta\theta$) is evident in Figs 1 and 2.

All measurements were made on the east-ring ansa, such that the tip of the A ring was approximately centred in the aperture. Thus, portions of both the A and B rings were included in the aperture, with A contributing about twice as much as B to the measured flux, at this inclination of the ring plane relative to the line of sight. Sky measurements were made by offsetting the aperture to the north, by about one aperture diameter, around the arc of a circle centred on Saturn's disk. Even on nights of

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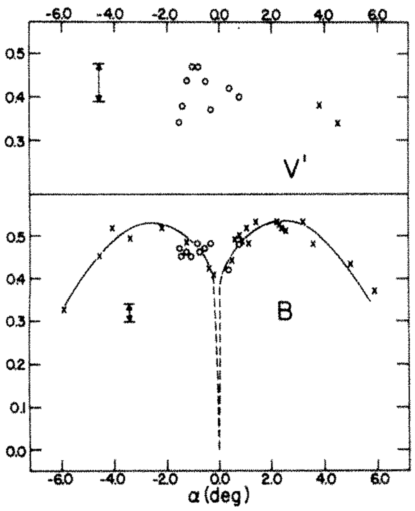

Fig. 1 Polarisation of Saturn's rings in B and V′ filters versus solar phase angle (α). ×, PMO data; O, KPNO data. Error bars at the left represent the typical error of one observation. A curve, symmetric about the origin, is hand drawn through the B-filter data. The dashed lines indicate an hypothesised polarimetric opposition effect. The position angle of polarisation always lies in the scattering plane, thus all the p values are 'negative'.

worst seeing, scattered light so measured never amounted to more than 10% of the flux on the ansa, either in terms of the polarised or total fluxes. Thus, stray light from Saturn's disk or other sources had no appreciable effect on the results.

Figures 2 and 3 show that the angle of polarisation always lies in the Sun–planet–Earth (SPE) plane. Because Saturn's orbital plane is slightly tilted out of the ecliptic plane, near opposition the SPE plane rotates by almost 180° on the plane of the sky. The polarisation follows the SPE plane, rotating also by almost 180°—see Fig. 3: this is our key result.

The tight correlation of the position angles with the position of the SPE plane, and the smoothness of our polarisation-phase curve over weeks, do not suggest any randomly varying component in the B-band polarisation as large as 0.10%. J.C.K. and Murphy[8] did not detect either azimuthal or temporal variations in the angle of polarisation around either the A or B rings to within ±4°. Comparing our measurements (at a ring tilt of −6°) with these (at a ring tilt of −26°), we also find no evidence for variation of the B-band polarisation with ring tilt, to within $\Delta p = \pm 0.1\%$, if comparison is made at the solar phase angle of 5.5°. The polarisation seems to depend only on the SPE (solar-phase) angle, at least in blue light.

Discussion

Because the angle of polarisation coincides strictly with the SPE plane, the polarisation seems to be mainly generated by intraparticle scattering (local scattering on individual, rough-surfaced ring bodies) and not by interparticle scattering (between discrete ring bodies).

Two other proposed mechanisms for the polarisation of Saturn's rings cannot explain the position-angle variation: (1) light received from the disk of Saturn and subsequently scattered towards us by the rings, or (2) multiple scattering between ring bodies, particularly in a monolayer.

Mechanism (2) here would be analogous to the mechanism which generates the phase-independent 'limb' polarisation which occurs on Jupiter's disk[10]. In that case the polarisation arises from double scattering between aerosol particles in a horizontal thin layer in the jovian atmosphere. This effect is shown in Fig. 4.

In a monolayer, a fraction of the incident sunlight is scattered between ring bodies in paths which lie parallel to the ring plane. Light could be scattered by one ring body (O) to bodies in front of or behind O, that is, a or a'; or to bodies on either side, that is, b or b'. But scattering to bodies above or below the ring plane (as c or c' in Fig. 4) is absent. This asymmetry results in a net polarisation, even with exact back-reflection, that is, at opposition. The dominant contribution to the polarisation is from the paths from O to b and b', which involve scattering angles of the order of 90° and thus large polarisations. As the polarisation at such angles for solid bodies has a positive sign, the resulting polarisation for the monolayer would no doubt be positive, with the emergent **E** vector normal to the monolayer.

In a low-density ring (many bodies thick), light paths c and c' in Fig. 4 are not eliminated. Then the interparticle scattering will tend to be isotropic, and the process just discussed would be suppressed.

We can set an upper limit to the polarisation contributed by double scattering between ring bodies in a monolayer model by assuming the rings to be a close-packed array of spheres, appropriate for the B ring which was an optical depth close to unity[4]. We write

$$p \approx GL \qquad (1)$$

where G is the ratio of doubly-to-singly scattered light, and L is the polarisation of light reflected by an individual ring body at a scattering angle ~90°. Higher-order scattering is ignored. G can be viewed as simply that portion of the sunlight impinging on one ring body which is intercepted by a second body and which, also, can then be deflected towards the Earth without shadowing. For an isotropic scattering phase function we find:

$$G \approx a\tilde{\omega}/(4\pi d^2) \qquad (2)$$

where $\tilde{\omega}$ is the equivalent single-scattering albedo for a ring body, d is the mean length of the scattering path between two ring bodies, and a is the mean area of all other ring bodies 'seen' by both the initial scatterer and the observer, taking account of the blocking by other ring bodies. $d = D - R$, where D is the mean centre-to-centre separation of ring bodies and R is the mean ring-body radius.

For the data presented here, the rings are seen close to edge-on. For a close-packed monolayer, only the tops of the individual bodies can be seen from the Earth or the Sun. The rays involved are those striking the top or 'cap' of one sphere, then being scattered by the exposed cap of an adjacent sphere, or more precisely by half of such an exposed cap. From a careful consideration of this geometry we find that at a ring-tilt angle of

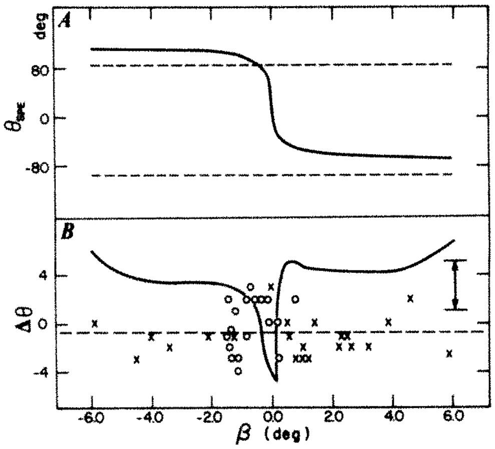

Fig. 2 *A*, The variation of the position angle of the Sun–planet–Earth plane (θ_{SPE}) with β, the angular distance of Saturn from the sub-solar point, along the ecliptic. (Note that $\beta \cong \alpha$, where α is the Sun–planet–Earth angle, except at very small α.) The dashed lines indicate the geocentric position of the major axis of the rings. *B*, The deviation of θ (the polarisation position angle) from θ_{SPE} (where $\Delta\theta = \theta - \theta_{\mathrm{SPE}}$). ×, PMO data; O, KPNO data. The solid line is the combined result of the measured mean curve (dashed line) with addition, in Stokes-vector space, of a hypothesised phase-independent polarisation of 0.08% having its **E** vector normal to the ring plane.

6°, with $\tilde{\omega} = 0.75$, the parameter G is quite small, about 0.01. This applies to the B ring. For the A ring, the ring optical depth being less by a half, the G factor would be also halved.

Thus the effect of a possible 'monolayer' constituent in our observed polarisation can be dealt with if we retain only L (the polarisation of a ring body at scattering angles ~90°) as an unknown parameter. A monolayer polarisation would be essentially phase-independent; the **E** vector would remain normal to the ring plane and would not follow the rotation of the SPE plane on the sky, as in Fig. 3. The observed angle behaviour is more consistent with a constant $\Delta\theta = 0$, that is, with the dashed line at the bottom in Fig. 2. The addition of a small monolayer effect would be to shift the curve $\Delta\theta$ from the zero line—we would find a perturbed curve $\Delta\theta(\alpha)$. Such a function is plotted (solid curve) in Fig. 2, for an arbitrary choice of $L = 8\%$. From least-squares fitting of $\Delta\theta(\alpha; L)$ to our position-angle data, we find that the 3σ upper limit for a monolayer-like polarisation is 0.03%. With $G = 0.01$ as estimated above, we deduce that the 90° polarisation by an individual ring body could not exceed 3%—if the rings were indeed a monolayer.

A similar disturbance $\Delta\theta(\alpha)$, (a variable change in position angle away from the SPE plane) would arise from a polarisation constituent due to light from Saturn's disk, rescattered to Earth by the rings. To discuss this we relax the assumption of a monolayer for the rings. Equation (1) applies, but G is now the ratio of sunlight scattered first by Saturn's disk and then by the rings, to sunlight returned directly to Earth by the rings. A fair value for G is ~0.01, as has been computed by Price and Baker[13] in another context. This polarisation would again be almost phase-independent, with the emergent **E** vector most likely normal to the ring plane. For estimating the magnitude of this effect we assume only single scattering of light by individual ring bodies; we neglect multiple light paths between discrete ring bodies. (Multiple scattering locally on the surface of a rough-surfaced ring body, however, is not ignored; that is included by specifying a value of L for a typical, macroscopic ring body.) Following equations of Cook *et al.*[14], we find that the ratio of multiply-to-singly scattered radiation from the rings, in this sense, does not exceed 0.1, for a low-density ring and a single-scattering albedo of 0.75; single scattering by the ring bodies is dominant. As for the pure monolayer effect discussed above, we are again able to place a 3-σ upper limit on the large-angle ring-body polarisation of ~3%; for a larger value, we would surely see a distortion in the $\Delta\theta$ curve.

Note that, regardless of the precise structure of the rings, our phase curves—especially of the position angle—provide an extrapolated upper limit for the polarisation, at angles of the order of 90°, for scattering from the ring bodies, namely about 3%. The small large-angle polarisation, the moderately large negative polarisation at small angles (~0.5% in the blue), and the flatness of $p(\alpha)$ near opposition, are unique. Only the published polarimetry data of a few laboratory samples are similar. They include a ground, transparent, amorphous silicate, uncontaminated by low-albedo material (contaminated silicates are not similar)[15]; and fine-grained water frost as well as freshly chipped white clay[16]. Thus the polarisation is particularly consistent with frost.

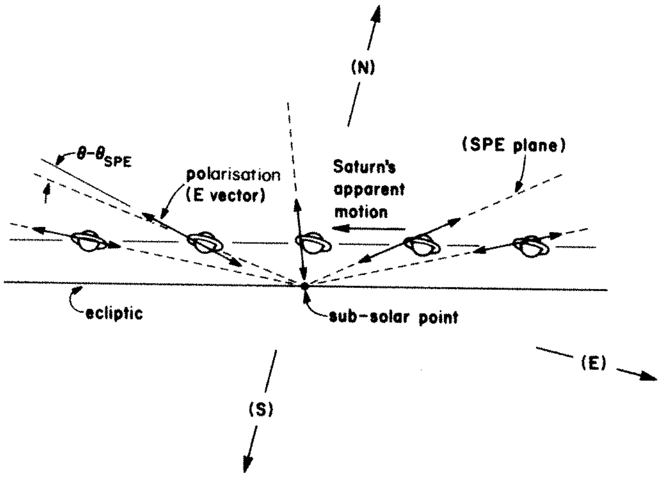

Fig. 3 A representation of the relationship between the position of Saturn's rings and the position angle of the ring polarisation. (Not drawn to scale.)

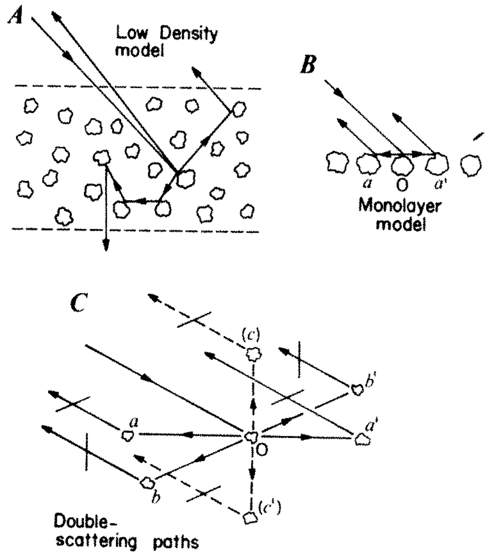

Fig. 4 The low-density and monolayer ring models showing the possible distribution of multiple scattering paths. In *C* particles *a* and *a'* are 'in front of' and 'behind' particle O, respectively. Particles *b*, *b'* are 'off to the side'; particles *c*, *c'* are 'above' and 'below' particle O respectively. In a monolayer, *c* and *c'* are absent. A monolayer viewed from the side is seen in *B*.

Both experimental evidence[7] and theoretical analysis[17] indicate that negative polarisation (**E** vector parallel to the scattering plane) cannot be generated by scattering between widely-separated particles. The polarisation phase curve of Saturn's rings is therefore apparently accounted for strictly by local scattering on individual rough-surfaced bodies, as with the Moon's polarisation; it is not controlled by effects of mutual shadowing and reflection between ring bodies.

Simple rough-surface polarisation should drop to zero at zero solar phase angle. Our phase curves, however, show only a slight trend to zero near opposition, even down to the minimum observed phase angle of 0.22°. The drop to zero must occur in a narrow 'cusp' <0.5° wide, for visible wavelengths. This very sharp opposition effect can also be seen in data for both the rings and for high-albedo laboratory samples, reported by Dollfus[16].

This behaviour agrees qualitatively with the theory of Wolff[17] for the polarisation of light reflected from rough surfaces. In his theory the small-angle polarisation is governed by double reflections between grain facets, combined with shadowing. He predicts a 'cusp' in the polarisation phase curve with a width of the order of that of the photometric ('brightening') effect at opposition. The known width of the latter is indeed <0.5° (ref. 1). We assume here that the photometric phenomenon is also due to localised scattering and shadowing on the rough surfaces of macroscopic ring bodies, rather than to mutual effects between such bodies.

Conclusion

In other astronomical contexts, for example, in B-emission stars, the presence of a disk-like structure is known or presumed to produce an optical polarisation, correlated in direction with the disk plane. In the most striking example of a disk-like structure in our Solar System, Saturn's rings, we have shown that such an effect is not readily visible. Rather, a competing effect, correlated geometrically not with the disk but with the Sun–planet–Earth plane, is dominant under all conditions, even near exact opposition, at which time the disk-like component should be most readily distinguishable. At least two mechanisms have been cited for the production of a disk-like polarisation: a monolayer effect; and the rescattering of sunlight reflected from the body of planet, by the rings. The former would operate only to the extent that the disk approximates a monolayer; but the second mechanism is surely applicable. The observed smallness of the contribution to the polarisation due to either or both of these mechanisms, $\leq 0.1\%$ in blue light, relative to the polarisation of $\sim 0.5\%$ caused by direct scattering of sunlight by the rough surfaces of individual ring bodies, has been attributed to the optical properties of the ring bodies. We conclude that the large-angle ($\sim 90°$) scattering polarisation of the ring bodies is quite small, $\ll 10\%$. This quantitative feature has evidently precluded the detection of a disk-like polarisation. Polarimetric results from Pioneer 11 will be of great interest, specifically with respect to our prediction that the large-angle polarisation of the rings—not directly measurable from Earth—must be very small.

Some investigators[14,18] have supported the hypothesis that the photometric opposition (brightening) effect is caused by shadowing within the surface microstructure of individual ring bodies. Others[19] have argued that the singular magnitude and sharpness of the effect is due mainly to mutual shadowing among the discrete ring bodies, and the opposition brightening by such a mechanism has been extensively modelled[12,19,20]. As the cusp in the $p(\alpha)$ curve (Fig. 1) is as narrow as the brightness peak, we can presume the polarimetric and photometric opposition effects to be parts of the same process; thus our results, substantiated by Dollfus' data, indirectly support the first of the two hypotheses for the brightening. If the polarisation were controlled by multiple light paths between the ring bodies, rather than by the local rough-surface texture, a ring plane-correlated polarisation would have been detected.

The polarisation phase curves for the rings are rather unusual when compared with those of other planetary objects and laboratory samples. Only a few published cases are known of laboratory samples exhibiting a strong, flat negative branch combined with a very weak positive polarisation at large angles. This may be due to the lack of laboratory data on high-albedo materials, especially frosts. More work on laboratory frosts would be of interest and should include the difficult polarimetric measurements at extremely small phase angles ($<0.5°$), so that the narrow 'cusp' can be defined.

Finally, more complex models for the rings may be necessary to account for all of the optical phenomena, including the polarisation behaviour. For example, a two-component model involving a haze layer is being considered in connection with the IR emission (R. E. Murphy, personal communication).

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Note added in proof: Larry Pleskot (private communication) finds that the sharp opposition effect of Saturn's rings can be approximated in the laboratory with a fine-grained winter frost covering a macroscopically rough substrate. Indeed, one characteristic of high-albedo surfaces seems to be such an opposition effect. He concludes, as we do, that the opposition effect of Saturn's rings cannot be modelled solely on the basis of mutual shadowing of discrete ring bodies, but must include the effects of scattering on the surface of individual bodies.

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U–Pb, Sm–Nd and Rb–Sr systematics of mid-ocean ridge basalt glasses

R. S. Cohen*, N. M. Evensen, P. J. Hamilton & R. K. O'Nions*

Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York 10964

The measurement of Pb, Nd and Sr isotopes in basalt glasses from mid-ocean ridges reveals correlations in isotope parameters which have important implications for the differentiation history of the mantle.

THE isotopic compositions of Pb, Nd and Sr in oceanic basalts reflect the fractionation histories of U/Pb, Sm/Nd and Rb/Sr in their mantle sources, and therefore may throw light on several important problems including the differentiation of mantle material to produce continental crust, the recycling of continental material and the degree of convective mixing in the mantle. As mid-ocean ridge basalts comprise nearly 80% of the Earth's volcanic products they should convey a good impression of the isotopic characteristics of the sub-oceanic mantle. However, ocean island basalts because of their accessibility and state of preservation have attracted much more attention from isotope geochemists despite their comparatively small volume.

Early investigations of the Rb–Sr systematics of oceanic basalts established that mid-ocean ridge basalts often possess Rb/Sr ratios that are too low to generate their measured $^{87}Sr/^{86}Sr$ ratios in ~ 4.5 Gyr from a chondritic initial $^{87}Sr/^{86}Sr$ ratio and further that significant differences exist in $^{87}Sr/^{86}Sr$ ratios from one mid-ocean ridge location to another (see refs 1, 2). The first reliable Pb-isotope compositions obtained from mid-ocean ridge basalts by Tatsumoto[3] showed that the evolution of their source had not involved a large fractionation in U/Pb ratio relative to the assumed whole Earth value. The available data base for Sr- and Pb-isotopes in mid-ocean ridge basalts has recently increased substantially[4–10], but only rarely

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* Present address: Department of Mineralogy and Petrology, University of Cambridge, Downing Place, Cambridge, UK.

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Table 1 Isotopic analyses of basalt glasses

Sample	Long.	Ref.	^{206}Pb§ / ^{204}Pb	^{207}Pb§ / ^{204}Pb	^{208}Pb§• / ^{204}Pb	U^{238} / Pb^{204}	^{87}Sr† / ^{86}Sr	^{87}Rb / ^{86}Sr	^{143}Nd‡ / ^{144}Nd	^{147}Sm / ^{144}Nd	Sm*	Nd*	Rb*	Sr*	U*	Pb*
A37.18N	35°12′ W	3	19.20 ±.03	15.59 ±.03	38.61 ±.10	6.2	0.70321 ±5	0.105	0.51309 ±3	0.226	2.46	6.55	2.27	62.5	0.060	0.625
A22.59N	43°31′ W	4	18.20 ±.02	15.46 ±.03	37.56 ±.05	9.64	0.70253 ±5	0.028	0.51314 ±3	0.209	3.42	9.38	1.88	194	0.063	0.408
A45.15N	28°.02′ W	2	19.28 ±.02	15.54 ±.02	38.83 ±.05	37.9	0.70314 ±4	0.126	0.51309 ±3	0.183	2.79	9.15	4.58	105	0.195	0.332
A11.16N	43°.39′ W	5	18.34 ±.02	15.47 ±.01	37.68 ±.03	4.81	0.70249 ±4	0.015	0.51312 ±3	0.208	4.13	11.92	0.66	123	0.043	0.559
AO5.47S	11°25′ W	6‖	17.84 ±.02	15.47 ±.02	37.33 ±.05	8.0	0.70230 ±4	0.032	0.51329 ±2	0.208	4.04	11.70	1.48	133	0.118	0.91
A59.46N	29°48′ W	1	18.31 ±.03	15.53 .03	37.90 ±.07	12.4	0.70279 ±4	0.032	0.51323 ±3	0.239	2.42	6.08	0.77	70.0	0.041	0.211
P00.42N	85°30′ W	8	18.56 ±.02	15.49 ±.02	38.07 ±.04	22.9	0.70275 ±5	0.132	0.51308 ±3	0.187	7.85	25.2	3.35	73.0	0.231	0.636
P44.40N	130°.30′ W	7	18.43 ±.03	15.44 ±.03	37.64 ±.06	17.6	0.70253 ±4	0.045	0.51313 ±3	0.200	4.86	14.6	1.79	114	0.137	0.502
P08.16S	108°.50′ W	9	18.46 ±.02	15.48 ±.02	37.97 ±.04	5.01	0.70265 ±5	0.007	0.51302 ±2	0.185	3.13	10.19	0.392	156	0.039	0.491
I50.25S	131°.00′ E	11	18.75 ±.02	15.49 ±.02	38.19 ±.05	12.5	0.70261 ±4	0.034	0.51305 ±2	0.180	4.77	15.9	2.11	180	0.134	0.681
I14.45N	54°48′ E	10	18.43 ±.03	15.43 ±.03	38.01 ±.06	6.3	0.70278 ±5	0.013	0.51302 ±2	0.192	1.84	5.77	0.35	78.4	0.023	0.230

Source of samples: (1) Reykjanes Ridge P601; (2) 45°N 56 (from F. Aumento); (3) DSDP 335–5–2, 5; (4) DSDP 396–18–3, 58; (5) Vema Fracture Zone 111327 (ref. 25); (6) AD3–3 (ref. 40); (7) Juan de Fuca Ridge 11240/60 (ref. 39); (8) Galapogos Ridge P115 (from W. G. Melson) described in ref. 41; (9) East Pacific Rise P6707–40 (from E. Bonatti); (10) Gulf of Aden 24B (from E. Bonatti); (11) Vema 33 Dredge (Lamont-Doherty Geological Observatory).

* All concentration data in μg per g.

† $^{87}Sr/^{86}Sr$ ratios expressed relative to $^{87}Sr/^{86}Sr = 0.70800$ for Eimer and Amend $SrCO_3$. Measured value for this standard is 0.70806.

‡ Normalised to $^{146}Nd/^{144}Nd = 0.7219$.

§ Data from NBS-981 indicates a fractionation of -0.6 ± 0.2‰. AMU^{-1}. No correction made.

‖ U and Pb concentration data from ref. 22.

have Pb- and Sr-isotopes been measured on the same samples. In the past few years Nd-isotope compositions have been obtained on mid-ocean ridge and oceanic island basalts[11–14] and in those instances where Sr-isotope compositions have been reported for the same samples there is a clear negative correlation between $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios. This correlation attests to a strong coherence in the fractionation of Rb/Sr and Sm/Nd during the evolution of the basalt sources. A similar comparison of the behaviour of U/Pb with Rb/Sr and Sm/Nd in oceanic basalt sources is more difficult, but the compilation of Pb- and Sr-isotope data made by Sun and Hanson[15] suggests that overall no such simple coherence exists. Further evaluation of these relationships has been hampered by the fact that Pb-, Nd- and Sr-isotope compositions have not been obtained on the same samples. An additional complication is that both Rb/Sr and $^{87}Sr/^{86}Sr$ ratios of ocean floor basalts may be modified by alteration processes operating on the sea floor. However, it has been shown that the glassy rims of pillow basalts generally provide the most reliable estimates of Rb/Sr and $^{87}Sr/^{86}Sr$ (ref. 2) and that the U-abundances of submarine basalts may be modified by interaction with seawater[16,17]. The reliability of many of the published Rb–Sr and U–Pb data on submarine crystalline basalts are thus very difficult to evaluate. The present study aimed to improve the existing situation by measuring $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$, $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios together with the abundances of U, Pb, Sm, Nd, Rb and Sr all on the same samples of mid-ocean ridge basalt glasses from the Atlantic, Indian and Pacific oceans.

Analytical techniques

To minimise the possibility of contamination, glass rather than crystalline material was carefully handpicked from drilled or dredged basalt samples. Typically an aliquot of glass between 50 and 150 mg was dissolved and Pb separated electrochemically using a miniaturised version of the technique described by Arden and Gale[18]. Sr and Nd were subsequently isolated from the samples using cation and anion exchange techniques respectively[12]. U, Pb, Sm, Nd, Rb and Sr abundances were determined on a second aliquot of glass by mass spectrometric isotope dilution analysis. The purification techniques used were the same as the above with the addition of a reverse phase

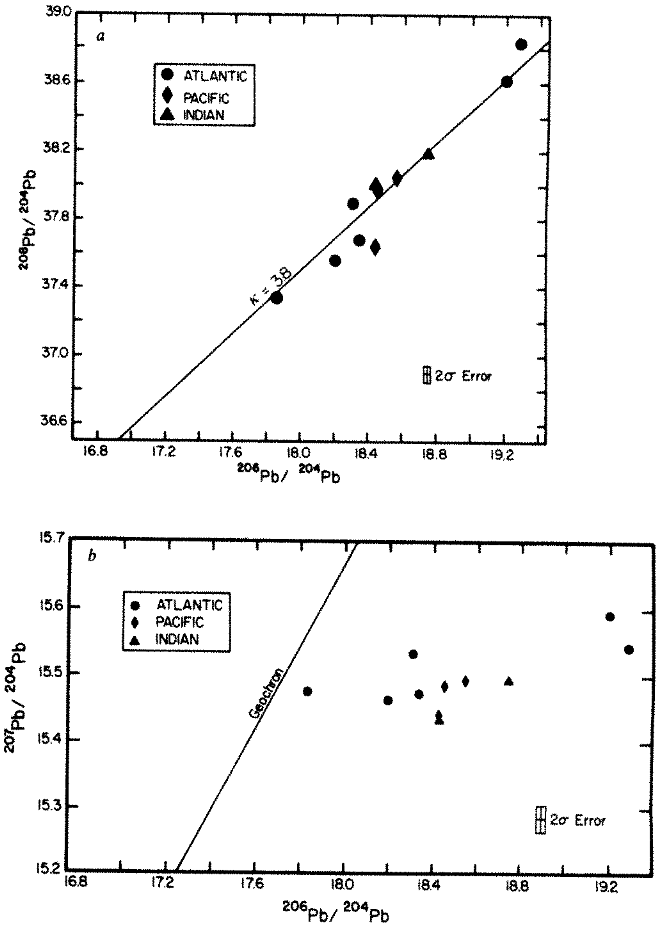

Fig. 1 *a*, Plot of $^{208}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ for basaltic glass samples from the Atlantic, Indian and Pacific oceans. The line drawn is for a value of κ ($^{232}Th/^{238}U$) = 3.8. The errors indicated are average errors (Table 1). *b*, Plot of $^{207}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ for basaltic glass samples from the Atlantic, Indian and Pacific oceans. The geochron has been constructed for 4.55 Gyr assuming initial Pb isotope compositions of Canyon Diablo[42]. The errors indicated are average errors for the analyses (see Table 1).

chromatographic procedure for the separation of U (ref. 19). The mass spectrometric techniques for the isotopic analysis of Pb, Rb, Sr, Sm and Nd have been described previously[12,20] and U was isotopically analysed using a triple-filament technique and ion-counting with a Daly detector. Procedural blanks are as follows: $U = 0.01$ ng; $Pb = 0.5$ ng; $Sm < 0.05$ ng; $Nd < 0.05$ ng; $Rb = 0.05$ ng; $Sr = 0.1$ ng.

Major element analyses of the glasses were obtained using an electron probe with energy dispersive analysis at the Department of Mineralogy and Petrology, Cambridge.

Results

The $^{208}Pb/^{204}Pb$, $^{207}Pb/^{204}Pb$, $^{206}Pb/^{204}Pb$, $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios together with U, Pb, Sm, Nd, Rb and Sr concentrations for 11 mid-ocean ridge basalt glass samples are given in Table 1 and their major element compositions are listed in Table 2. The sample numbering system in Tables 1 and 2 is similar to that used by Melson *et al.*[21] where the letter prefix (A, I or P) denotes the ocean concerned and the following sequence of numbers and letter denote the latitude. Although the data in Table 1 are not fully representative of the Earth's mid-ocean ridge basalts they are adequate to demonstrate the nature of the isotopic variations that exist.

The Pb-isotope compositions for all of the samples are compared on plots of $^{208}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ in Fig. 1*a*,*b*. The $^{207}Pb/^{204}Pb$ and $^{206}Pb/^{204}Pb$ ratios of the samples all plot to the right of the geochron (4.55 Gyr isochron) and exhibit a positive correlation similar to that noted previously for mid-ocean ridge basalts[7,22]. The range of Pb-isotope compositions in the Atlantic samples is greater than that for either the Indian or Pacific oceans, but this may only reflect the limited size of the sample set.

$^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios are compared with the trend previously defined by mid-ocean ridge and ocean island basalts in Fig. 2. The samples exhibit a considerable range in both $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ ratios, and conform closely to the previously documented trend for oceanic volcanics. The highest $^{87}Sr/^{86}Sr$ ratios are found in basalt glasses from 37 °N and 45 °N on the Mid-Atlantic Ridge (A37.18N and A45.15N) close to the Azores platform (refs 10, 23). Conversely the sample with the lowest $^{87}Sr/^{86}Sr$ and highest $^{143}Nd/^{144}Nd$ ratio is from the Mid-Atlantic Ridge at 6 °S (A05.47S).

The most important new observation to be made about these data is probably the association of the least radiogenic Pb isotope and Sr isotope compositions and vice versa (Fig. 3). Overall there is a good positive correlation between the $^{87}Sr/^{86}Sr$ and $^{206}Pb/^{204}Pb$ which indicates a strong coherence between U/Pb and Rb/Sr during the evolution of the mid-ocean ridge basalt source region. Note that the Pb- and Sr-isotope data reported for Icelandic and Reykjanes Ridge basalts[8,24] are consistent with the correlation shown in Fig. 3, but in general, data reported from other oceanic islands do not show such a consistent correlation (see compilation in ref. 15).

The abundances of U, Pb, Rb, Sr, Sm and Nd in the basalt glasses show considerable variation (Table 1), for example, Rb/Sr ratios vary by more than an order of magnitude from 0.003 to 0.044 and U/Pb ratios range from 0.077 to 0.587. With the exception of sample A37.18N the Rb/Sr and U/Pb ratios are positively correlated (Fig. 4). A second and apparently pure 5-mg aliquot of A37.18N has been analysed and found to have $Rb/Sr = 0.065$, a factor of two higher than the first sample. The reason for the highly variable Rb abundance in this glass is not understood. Correlations between $^{87}Rb/^{86}Sr$ and $^{87}Sr/^{86}Sr$ and $^{238}U/^{204}Pb$ and $^{206}Pb/^{204}Pb$ ratios respectively are poor as seen in a plot of $^{238}U/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ in Fig. 5 and do not provide any evidence for discrete mantle fractionation events.

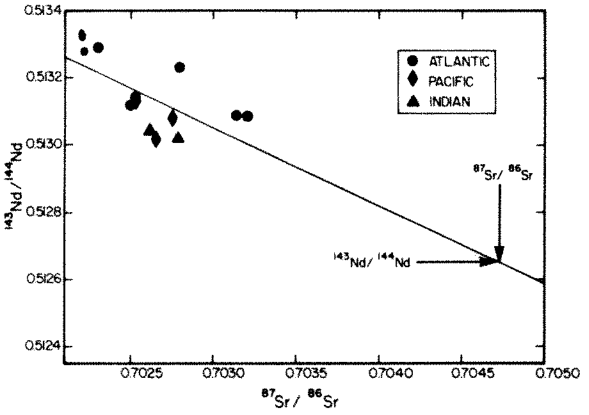

Fig. 2 Comparison of $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios of basalt glasses from the Atlantic, Indian and Pacific oceans with the trend defined previously for oceanic volcanics[11-13]. The arrows indicate the estimated $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios of the bulk Earth[12,13].

The major element analyses of the samples (Table 2) are typical of those for mid-ocean ridge tholeiites (see, for example, ref. 25) with SiO_2 contents ranging from 49.12 to 52.47 wt %. None of the major element abundances or ratios such as $MgO/(MgO+FeO)$ show any clear correlation with trace element abundances, or abundance ratios such as Rb/Sr or U/Pb.

Discussion

The $^{143}Nd/^{144}Nd$ and $^{87}Sr/^{86}Sr$ ratios of the glasses are respectively greater than and less than the model bulk Earth values for these ratios and are consistent with the negative correlation identified previously for oceanic basalts[10-12]. It has been suggested that a unidirectional transport model involving the extraction of silicate melt from the mantle to leave a residuum with lower Rb/Sr and higher Sm/Nd ratio than the original would produce with time the Nd- and Sr-isotope characteristics of mid-ocean ridge basalts[12,26-28]. The general correlation between $^{206}Pb/^{204}Pb$ and $^{87}Sr/^{86}Sr$ ratios in mid-ocean ridge basalts suggests that there was a coherence between U/Pb and Rb/Sr during the evolution of the basalt source region. Is this correlation consistent with the simple unidirectional transport models that have been used to accommodate the Nd and Sr data? An assessment of this possibility is made difficult by uncertainties in the values of U and Pb distribution coefficients

Table 2 Major element analyses of MORB glasses

	A59.46N	A45.15N	A37.18N	A22.59N	A11.16N	A05.47S	P44.40N	P00.42N	P08.16S	I14.45N	I50.25S
SiO_2	52.47	52.20	50.90	49.68	50.06	49.48	51.54	49.85	49.12	51.32	50.90
TiO_2	1.10	0.98	0.98	1.18	1.45	1.39	1.88	3.26	1.37	0.87	2.12
Al_2O_3	13.54	14.75	15.06	15.25	15.76	16.72	13.47	11.69	16.82	15.30	15.25
FeO	12.03	9.47	9.02	8.43	9.99	8.62	11.63	16.72	9.00	9.44	10.02
MnO	0.15	—	—	—	0.17	0.19	0.10	0.09	—	0.08	0.16
MgO	6.83	9.13	7.75	7.97	7.87	8.20	6.73	4.86	8.58	8.57	8.02
CaO	11.83	11.31	11.51	11.01	11.13	11.14	11.00	9.25	10.76	12.22	10.27
Na_2O	2.21	2.04	2.39	2.47	2.79	2.66	2.67	2.49	3.24	2.35	2.89
K_2O	—	0.18	—	0.06	0.12	0.24	0.06	0.16	—	—	0.22
	100.16	100.06	97.61	96.05	99.34	98.64	99.08	98.37	98.89	100.15	99.85

Dash indicates element below level of detectability with counting times used. A05.47S from Engel and Engel (ref. 40). A11.16N from Melson and Thompson (ref. 25).

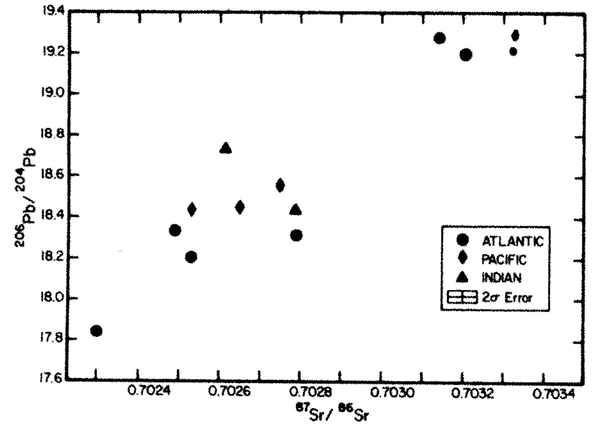

Fig. 3 Plot of $^{206}Pb/^{204}Pb$ versus $^{87}Sr/^{86}Sr$ for basaltic glasses from Atlantic, Indian and Pacific oceans. Average error is indicated.

in silicate systems, by the role of minor U- and Pb-bearing phases in the source region, and the uncertainty of the bulk Earth lead isotope composition. Whereas some ocean island basalts have $^{206}Pb/^{204}Pb$ ratios higher than any reasonable estimate of the bulk Earth Pb-isotope composition[29,30], the relationship of the Pb-isotope compositions of mid-ocean ridge basalts to the bulk Earth is less certain. If the bulk Earth Pb-isotope composition lies on the geochron (plotted for an assumed of 4.55 Gyr) in Fig. 1*b*, as is generally assumed, then all of the ocean ridge basalts under discussion here have $^{206}Pb/^{204}Pb$ ratios higher than the bulk Earth value. If the previous interpretations of the Nd and Sr-isotope correlation in oceanic basalts are applied to the above Pb- and Sr-isotope data, then those samples with highest $^{87}Sr/^{86}Sr$ ratios are considered to have been derived from less depleted (in a time-integrated sense) mantle than samples with lower $^{87}Sr/^{86}Sr$. Extrapolation of the $^{206}Pb/^{204}Pb$ versus $^{87}Sr/^{86}Sr$ correlation to a model $^{87}Sr/^{86}Sr$ ratio of the bulk Earth (~ 0.7050) would suggest that the $^{206}Pb/^{204}Pb$ ratio of the bulk Earth is ~ 21.3, a much greater value than other estimates of the bulk Earth Pb-isotope compositions. Furthermore if a unidirectional transport process effected a decrease in Rb/Sr and a concomitant increase in U/Pb in the residual mantle, then the lowest $^{87}Sr/^{86}Sr$ ratios would be associated with the highest $^{206}Pb/^{204}Pb$ ratios—the opposite to what is observed! Clearly a more complex model of Earth evolution than a simple unidirectional transport model, is required to accomodate the Pb-, Nd- and Sr-isotope data.

At this stage several more complex models should be considered. For example, it has been suggested[31,32], that Pb may have been partitioned into sulphide phases preferentially to U and subsequently located in the core during Earth differentiation. If, for example, such a process increased the U/Pb ratio in some part of the mantle, but not the Rb/Sr ratio, then at present it would have a $^{87}Sr/^{86}Sr$ ratio of 0.705 (the bulk Earth value) and a $^{206}Pb/^{204}Pb$ ratio in excess of the bulk Earth value. To produce the correlation in Fig. 3 this source or magmas derived from it would have to mix with those from another mantle source that had suffered a reduction in Rb/Sr ratio but comparatively little change in U/Pb ratio. Alternatively other workers have used models involving bidirectional transport of material between the mantle and the continental crust[33,34] to generate the Pb- and Sr-isotope compositions of oceanic volcanics. Recently Doe and Zartman[35], O'Nions *et al.*[36] and Evensen *et al.*[37] have pointed out that the continental crust may be considered as two reservoirs as far as Pb-isotopes are concerned. Because U is preferentially concentrated in the upper parts of the continental crust, the upper crust evolves with a relatively more radiogenic Pb-isotope composition than the lower part. Any preferential recycling of upper crust into the mantle would result in a mean Pb-isotope composition of the mantle more radiogenic than the bulk Earth and could account for the observed range of Pb-isotope composition found in oceanic basalts. Whether or not all the features of Pb-, Nd- and Sr-isotope chemistry of mid-ocean ridge basalt glasses can be reproduced by such models of continent recycling must await the results of further computations. Nevertheless, adopting such models would implicitly favour a continuous rather than episodic evolution of Pb-isotopes in the mantle.

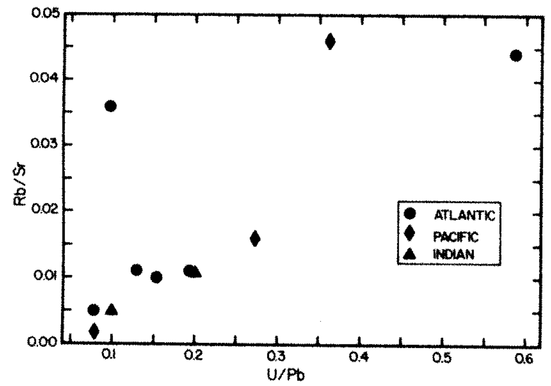

Fig. 4 Plot of U/Pb and Rb/Sr in basaltic glasses from the Atlantic, Indian and Pacific oceans.

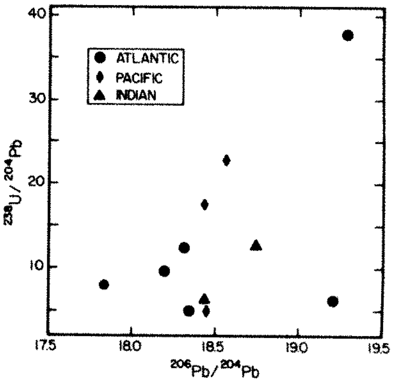

Fig. 5 Plot of $^{238}U/^{204}Pb$ versus $^{206}Pb/^{204}Pb$ for basaltic glasses from the Atlantic, Indian and Pacific oceans.

The comparatively small variations in Pb-, Nd- and Sr-isotope compositions in the glasses require that the time-integrated (over the duration of Earth history) Rb/Sr, U/Pb and Sm/Nd ratios in the various source regions must have been quite uniform. This is in marked contrast to the order of magnitude variation of Rb/Sr and U/Pb in the glasses. Such large variations could not be generated from a uniform source composition if the partial melting which produced the basalts followed a simple equilibrium melting model (for example see ref. 38) and the primary magmas produced underwent only small amounts of subsequent crystallisation differentiation. Thus it must be inferred either that the processes of basalt magma generation and evolution of mid-ocean ridges can effect much greater fractionation of Rb/Sr, U/Pb and Sm/Nd than is often appreciated or alternatively some mantle process effects large, but transient fractionation before the melting event. The petrogenetic utility of trace element abundances in oceanic basalts is severely limited until some resolution of these alternatives can be made.

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Tholeiitic basalt–rhyolite magmatism and massive sulphide deposits at Matagami, Quebec

P. J. MacGeehan[*] & W. H. MacLean

Abitibi Project, Department of Geological Sciences, McGill University, 3450 University Street, Montreal, Quebec, Canada H3A 2A7

Noranda-type massive sulphide deposits in some Archean greenstone belts of the Canadian Shield occur in tholeiitic volcanic sequences. The tholeiitic rocks belong to basalt–rhyolite bimodal suites with low Al_2O_3, high Na/K ratios, high iron contents, and well-defined iron enrichment trends.

IN Archean greenstone belts of the Canadian Shield Fe–Zn–Cu massive sulphide deposits of the Noranda-type are invariably described as occurring in calc-alkaline volcanic rocks[1–4]. Within each belt the volcanic succession typically consists of a laterally extensive sequence of tholeiitic basalt forming the lower two-thirds of the stratigraphy (Fig. 1*a*), overlain by volcanic rocks of calc-alkaline attribution (basalt, andesite, dacite and rhyolite) that form thick accumulations at discrete volcanic centres[1,5–7]. The accompanying massive sulphide deposits, ranging up to 2×10^8 tonnes in size (~5% Zn, 2% Cu), are composed mainly of pyrite, pyrrhotite, sphalerite and chalcopyrite, and occur as stratiform lenses clustered within the 'calc-alkaline' centres, most commonly on the flanks of rhyolite domes or other felsic accumulations. The sulphides were deposited as chemical sediments on the ancient seafloor[8], around or downslope from exhalation centres (now mineralised alteration pipes) that emitted hot metalliferous brines akin to those now found at the bottom of the Red Sea[9]. The massive sulphides thus form part of the original stratigraphy, and their apparent association with calc-alkaline rocks has historically made the latter an essential criterion for defining mineral exploration targets. However, in one of these large Archean volcanic centres, at Matagami, Quebec, this relationship does not hold; the massive sulphide deposits, while originally thought to lie within calc-alkaline rocks[10], are now shown to occur within a bimodal basalt-rhyolite suite of tholeiitic affinity[11,12]. Their apparent 'calc-alkaline' affinity is due to the widespread hydrothermal alteration that accompanied sub-seafloor geothermal activity, as will be discussed elsewhere. In this article we present evidence of the tholeiitic nature of these rocks, discuss their relationship to the ore deposits, and suggest that other Noranda-type massive sulphide deposits may occur in tholeiitic volcanic centres.

Geology of the Matagami district

The pertinent stratigraphy at Matagami consists of 80% basalt and 20% rhyolite (Fig. 1*b*,*c*). It contains over 18 mostly Zn-rich stratiform massive sulphide lenses, all closely associated with rhyolites. These rocks and ores have been intruded by the Bell River Igneous Complex and many of its subsidiary dykes and sills, then metamorphosed to the greenschist facies, and folded into a westward plunging anticline[13] (Fig. 2). The sulphide

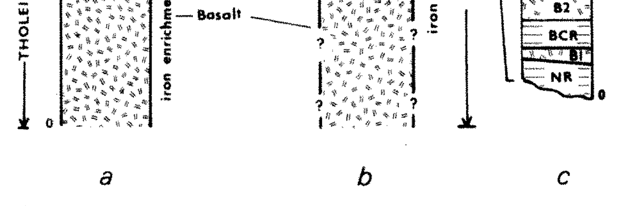

Fig. 1 Comparison of the stratigraphy in a typical Archean greenstone belt: *a*, to that at Matagami; *b*, with detail for Matagami in *c*. B1–B7 are basalts; NR, Norita rhyolite; BCR, Bell Channel rhyolite; GLR, Garon Lake rhyolite; FP, feldspar porphyry (andesite).

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[*] Present address: Geological Sciences, Queen's University, Ontario, Canada, K7L 3N6.

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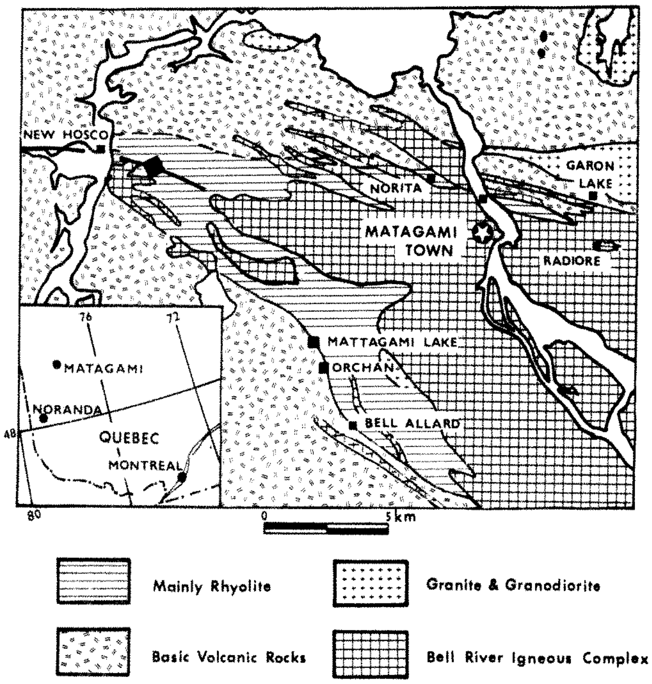

Fig. 2 Simplified geology of the Matagami district (after Sharpe[13]).

deposits, ranging up to 25 Mtonnes in size, occur within a 1 km thick stratigraphic section, and they now form two linear mineralised belts, one on either side of the anticline. On the south limb the deposits (Bell Allard, Orchan, Mattagami Lake, New Hosco) are all located at the base of the 'Key Tuffite'[14,15], a cherty tuffaceous unit that lies on top of a massive rhyolite, whereas those on the north limb are associated with rhyolites at several stratigraphic horizons: Norita rhyolite (Norita mine, Bell Channel No. 1), Bell Channel rhyolite (Bell Channel No. 4, Radiore), and Garon Lake rhyolite (Garon Lake mine)[11,12].

Stratigraphy

The volcanic rocks on the north limb of the anticline have been mapped and sampled in detail, and each unit has been analysed for major and several trace elements (Fig. 4). The stratigraphic sequence is composed of seven pillowed basalt units, a pillowed andesite and three rhyolite flows (Fig. 1*c*). The basalts are mostly aphyric or weakly plagioclase–phyric, but one unit, B2, is coarse grained throughout and contains abundant phenocrysts of plagioclase and pyroxene (now uralitised) in about equal proportions. The three rhyolite units are spherulitic flows which form domal accumulations up to 150 m thick, with associated pyroclastic material. All three units contain phenocrysts of quartz (inverted β-quartz) and sodic plagioclase, and rare granophyre xenoliths (Fig. 3*a*). The Bell Channel rhyolite also contains microphenocrysts of tridymite (inverted) (Fig. 3*b*)[12].

These volcanic rocks are intruded by concordant gabbro sills ranging from 50 to 250 m thick which grade laterally into underlying feeder dykes connected to the Bell River Igneous Complex. Contact features bordering these minor intrusions indicate they were emplaced contemporaneously with the extrusive rocks, and some indeed may represent lateral feeders to overlying flows[11].

Petrology and primary geochemical trends

All the volcanic rocks in the district have undergone a weak pervasive hydration, but alteration was more intense in the vicinity of the sulphide deposits; there, the rhyolites are chloritised and the underlying basalts are strongly spilitised and silicified. However, the stratigraphic sequence was extensively sampled for more than 5 km along strike, and the primary geochemical features of each rock unit were established (Fig. 4) using samples in which the primary mineral fabric was well preserved.

Basalt: the seven basalt units consist of plagioclase (saussuritised), pyroxene (uralitised), and minor opaque oxide set in devitrified glass that is either chloritic (B2–B4) or quartzofeldspathic (B5–B7). They are all quartz normative and have low Al_2O_3, high Na/K ratios, and high iron contents. Plots of the oxides against differentiation index[16] (Fig. 4) show minor fractionation of the units, but they all cluster at the basaltic end of the diagram, well separated from plots of the rhyolites. On an AFM diagram (Fig. 5*a*) the basalts (B2–B5) follow a progressive iron enrichment trend within the tholeiitic field, and this is re-emphasised on an MgO versus FeO* diagram (Fig. 5*b*; plots of Fe/(Fe+Mg) versus Si (refs 17, 18), FeO* or SiO_2 versus FeO*/MgO (ref. 19) similarly emphasise this relationship). Rocks of unit B5, which show maximum iron enrichment, first crystallised plagioclase and pyroxene, followed by magnetite, sodic feldspar, quartz and albite—the latter two minerals forming a spherulitic mesostasis to the other grains (Fig. 3*c*). This paragenesis provides strong petrographic support for a tholeiitic trend, and shows the transition from iron enrichment to alkali enrichment during the crystallisation of individual pillows.

Rhyolite: the three rhyolite units have high SiO_2 contents, but low Al_2O_3, high Na/K ratios, and high iron contents, like the basalts (Fig. 4). They plot at the alkali end of the tholeiitic field on an AFM diagram (Fig. 5*a*), but have normative plagioclase compositions around $An_{20}(An_{18.5-23})$ and are thus 'dacites' according to most rock classifications[20,21]. There is corroborating petrographic evidence that the plagioclase phenocrysts originally had this composition. All three units contain quartz and plagioclase phenocrysts and micrographic intergrowths (Fig. 3*a*), indicating they were crystallising on a cotectic in the Q–Or–Ab–An system before extrusion. However, in at least one unit (BCR), β-quartz was succeeded by tridymite after extrusion (Fig. 3*b*), yielding a minimum magma temperature of 867 °C (ref. 22) but probably as high as 935 °C (ref. 12). These temperatures are well above the liquids of melts crystallising albite, but close to that of melts crystallising plagioclase An_{20} in

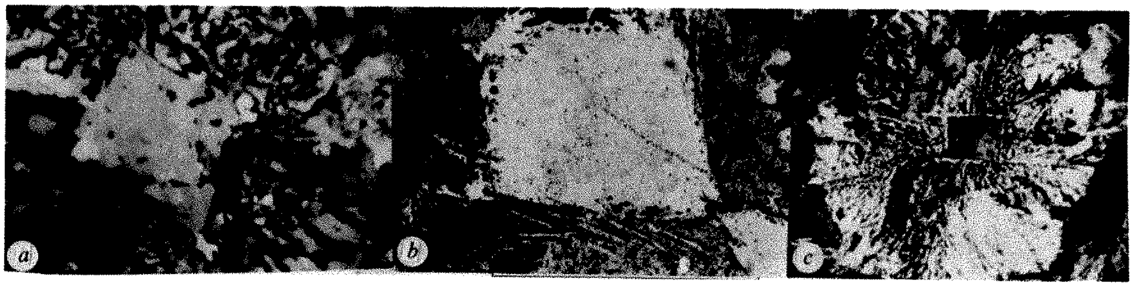

Fig. 3 *a*, From a granophyre fragment in the Garon Lake rhyolite; central crystal is inverted β-quartz surrounded by a micrographic intergrowth of quartz (white) and albite (grey-black). *b*, Tridymite phenocryst with reticulate borders, flanked by elongate tridymite microlites, now inverted to quartz (from the Bell Channel rhyolite). *c*, Spherulitic quartz–albite intergrowth mantling plagioclase and magnetite grains in the mesostasis of B5 basalt.

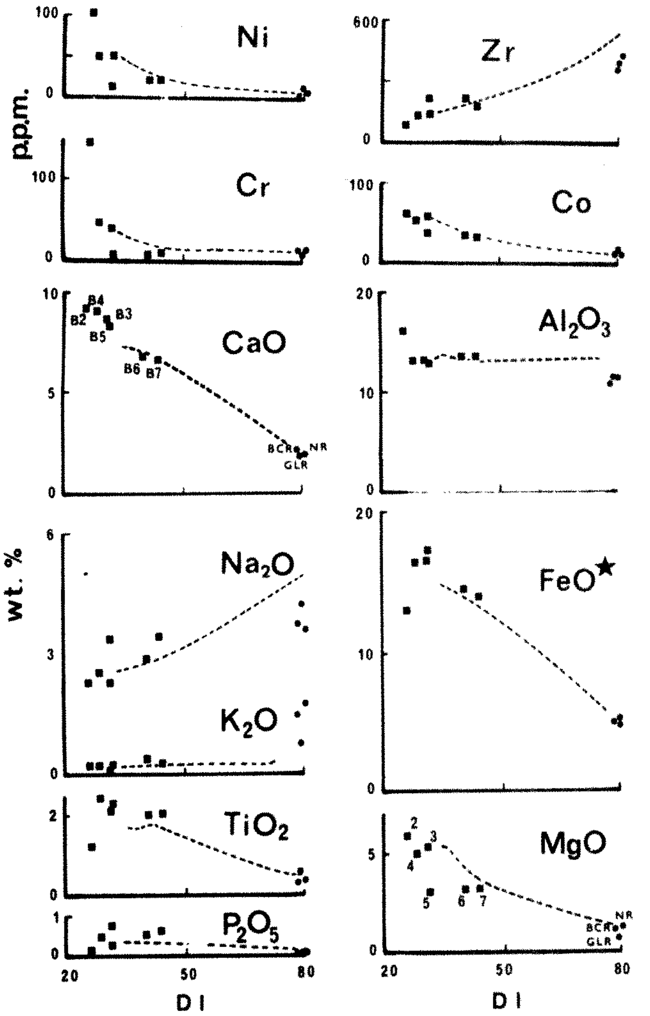

Fig. 4 Major oxides and trace elements[11,12] plotted against differentiation index (DI) show the bimodal distribution of the volcanic suite. ■, basalts; ●, rhyolites. The dashed line is the liquid-line-of-descent for the gabbro–granophyre sill[12] that was intruded contemporaneously with volcanism.

the quinary system Q–Or–Ab–An–H_2O at 2 kbar pressure[23,24]. As noted above, silicic rocks with these high normative An contents are generally classed as dacite, but our three units have SiO_2 contents >10% higher than equivalent dacites in Nockolds' classification[25]. Thus, although these rocks are actually dacites, we will follow the usage of other workers in the Canadian Shield for the time being who call these rocks rhyolite based on their high SiO_2 content[4,27,28] and the present albitic composition of the plagioclase phenocrysts.

Contemporaneous gabbro sills

The contemporaneous gabbro sills have compositions very similar to the basalts[12,13], and each sill fractionated along an iron enrichment trend. One highly fractionated gabbro-granophyre sill was studied in detail[12], and its chemical composition and fractionation trend (Figs 4, 5) place it well within the field of tholeiitic rocks, where it has an iron enrichment trend comparable with that of the well established tholeiitic suite from the Thingmuli Central Volcanic Complex in Iceland[18] (Fig. 5*b*). The trend in the liquid line-of-descent of this sill (dashed lines, Figs 4, 5) is believed to provide a good approximation of the probable fractionation path of the volcanic rocks at Matagami[12].

Interpretation

Using differentiation index as a monitor of progressive fractionation (Fig. 4) the aphyric basalts have chemical compositions essentially identical to successive liquids in the gabbro-granophyre sill (Fig. 4), and follow a comparable but more extreme iron enrichment trend (Fig. 5). Furthermore, each successive flow is more fractionated, indicating that the whole suite was derived from a common parent by progressive fractionation. This is supported by the trace element distributions in the basalts. Cr, Ni and Co have high concentrations in the earliest basalt flows, and decrease rapidly with fractionation, whereas Zr has a low initial value but becomes progressively enriched in the later flows. This is identical to their distribution in the gabbro–granophyre sill (dashed line, Fig. 4), and similar to that of Skaergaard[28], Thingmuli[29], and other tholeiitic suites. Preliminary studies on the REE content of the basalts and the sill indicate both have similar distribution patterns (Fig. 6*a*). These are typical of tholeiites in their low REE content and lack of LREE enrichment, and similar to those of modern oceanic basalts[30] and to that of the Skaergaard liquid after crystallisation of the Middle Zone[31]. Although highly fractionated, they also plot in the field of 'ocean floor' and 'within-plate' tholeiites on a Ti–Zr–Y diagram[32] (Fig. 6*b*).

The rhyolites and the granophyre of indicated tholeiitic affinity, have similar mineralogy and texture and almost identical composition (Fig. 4). However, both have significantly higher FeO*, MgO (and CaO), and higher temperature of crystallisation than the Thingmuli rhyolites (Fig. 5*b*), consistent with their calcic compositions. The granophyre represents a

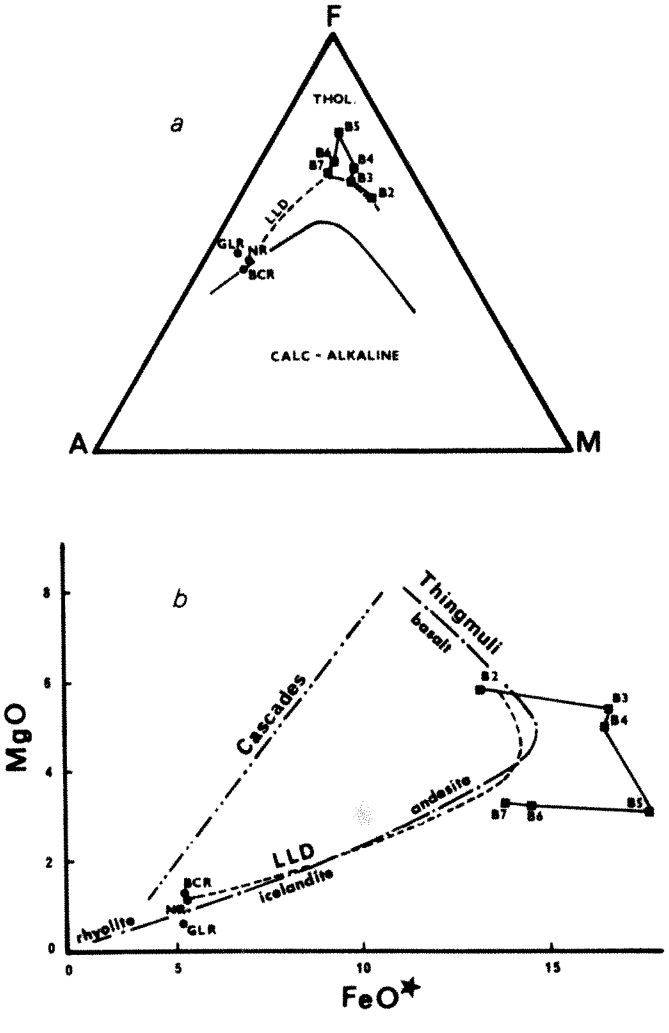

Fig. 5 *a*, AFM plot (wt%) of the volcanic units and the liquid-line-of-descent (LLD) of the gabbro–granophyre sill (dashed). The dividing line (solid line) for the tholeiitic and calc-alkaline fields is from Irvine and Baragar[20]. *b*, MgO versus FeO* (total iron) plot with comparison to Thingmuli (tholeiitic) and Cascades (calc-alkaline).

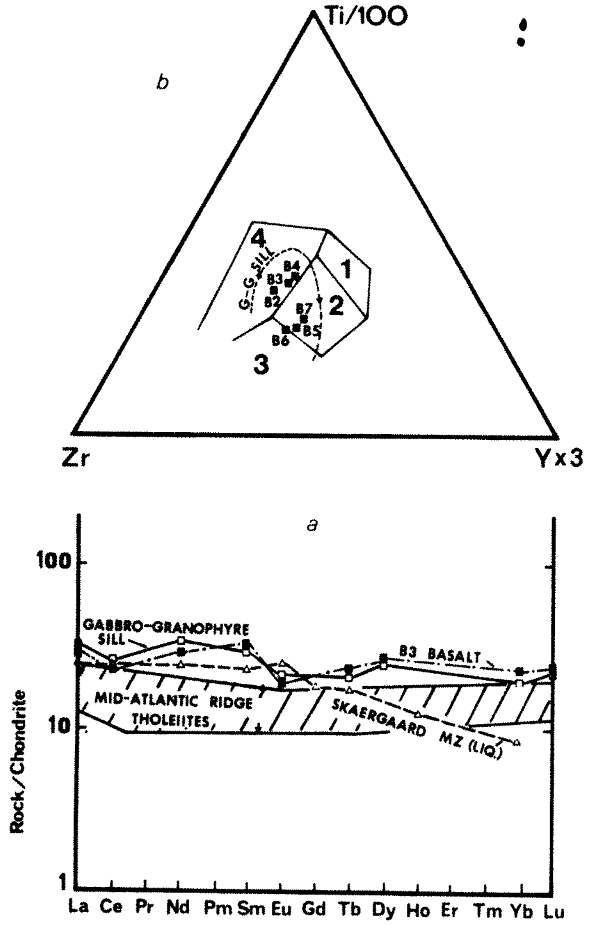

Fig. 6 *a*, Rare earth element analyses for B3 basalt and the gabbro–granophyre sill, compared to the middle zone (MZ) liquid of the Skaergaard intrusion[31] and the Mid-Atlantic Ridge tholeiites from the FAMOUS expedition[30]. *b*, Ti–Zr–Y plot[32] of basalts and sill. Fields **1** and **2** are low-K basalts; **2** is ocean floor tholeiite; **3** is calc-alkaline basalt; and **4** is within-plate basalt. Analyses for REE were by neutron activation at X-ray Assay Laboratories, Toronto, Canada, and chondrite-normalised using the values for the Leedey chondrite[46].

residual melt derived by fractionation of a parental magma very similar in composition to the basalts (dashed line, Fig. 4), leaving but little doubt that it and the rhyolites shared a common origin—as residual melts derived through progressive fractionation of a parental tholeiitic basaltic magma.

Preliminary studies on the underlying Bell River Igneous Complex indicate that its rocks too are very iron-rich[33], and the layered gabbro–anorthosite series is not disrupted by gabbro dykes and sills that transect the overlying rocks. We interpret these field and chemical relations to indicate a comagmatic source, with the Complex emplaced high into the volcanic pile (probably within 1 km of the surface), and itself acting as a holding chamber while supplying differentiated magma to overlying flows and sills.

Matagami lies at the western end of a linear belt of mixed tholeiitic volcanic rocks and layered gabbroic and anorthositic plutons which includes the Chibougamau mining district 250 km to the east[34]. In geologically younger parts of the Earth's crust, thick tholeiitic volcanic sequences with associated large basic layered intrusions are found only in areas of tensional tectonics[35], for example, Skaergaard, Duluth, and Scottish Tertiary complexes. The similarity of the younger examples to these older rocks suggests the Matagami–Chibougamau volcanic belt also formed in a tensional environment.

Implications

The ores at Matagami are typical Archean Noranda-type deposits being (1) polymetallic (Fe–Zn–Cu–Ag–Au) massive sulphides of volcanogenic exhalative origin, (2) stratiform in configuration, and (3) associated with rhyolites near the tops of thick sequences of essentially basic submarine volcanic rocks[36]. In form and origin they are identical to younger volcanogenic types, but in metal content or in type of associated volcanic rock they may differ[37,38]. The younger deposits are widely regarded as falling into two major classes: (1) Fe–Cu-rich ores in basalts of tholeiitic affinity in tensional tectonic settings (Cyprus-type: spreading ridge; Besshi-type: back arc), and (2) Fe–Zn–Cu–Pb ores in rhyolite and dacite of calc-alkaline affinity developed along consuming plate margins (Kuroko-type)[36,39,40]. Although Noranda-type deposits contain only minor or trace amounts of Pb, they have been compared most frequently with the Kuroko-type because of their association with rhyolites and the apparent calc-alkaline nature of the volcanic sequences. Consequently, geological thought and exploration strategy in shield areas have been unduly preoccupied with the 'calc-alkaline' association of these ores, and by analogy, with compressional zones in the Earth's crust.

However, genesis of these massive sulphide ores is intimately related to sub-sea floor geothermal activity[11,41–43] during which Fe, Mg, Cu, Zn and many other elements are leached from large volumes of the underlying volcanic rocks and deposited at the sediment–seawater interface. It is these hydrothermally altered volcanic rocks, whose $^{87}Sr/^{86}Sr$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ ratios[42,43] and major and trace element geochemistry[11,12] have been so strongly disturbed, that are often described as 'calc-alkaline'. The apparent 'calc-alkaline' affinity of hydrothermally altered tholeiitic volcanic rocks in many mining districts may thus be the inevitable consequence of sub-seafloor geothermal activity, as will be outlined elsewhere.

Thus, Noranda-type massive sulphide deposits do occur in association with tholeiitic volcanic rocks, both here in Matagami, in the Flin Flon–Snow Lake mining district[44], and apparently in several other districts[45]. This and other evidence also indicates they formed in tensional environments in the Earth's crust. Therefore, we conclude that sequences of submarine volcanic rocks containing rhyolites, whether of tholeiitic or calc-alkaline affinity, are prime exploration targets for Noranda-type massive sulphide ores.

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Genetic variance of laboratory outbred Swiss mice

M. Catherine Rice & Stephen J. O'Brien*

Laboratory of Viral Carcinogenesis, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

The extent of allelic variation has been estimated at 46 structural gene loci within three major colonies of Swiss mice and between inbred derivative strains. The colonies have retained nearly the same amount and type of variation found in natural murine or human populations despite laboratory propagation for more than 50 years (175 generations). The population genetic structures of the Swiss mouse colonies were comparable to an island population in which random fixation, and not inbreeding or population bottlenecks, is apparently responsible for slight losses in genetic variance.

RODENTS provide a general standard in toxicological and carcinogenesis testing[1,2] and laboratory mice (*Mus musculus*) are especially useful because they are inexpensive, available in large number and have short lifespans. A major advantage is the availability of numerous inbred strains homozygous for over 98% of their genetic loci. In addition, considerable genetic information about the differences between strains has accumulated from research on mouse genetics, oncology and toxicology[3-5]. However, the divergence between inbred strains is now a source of criticism of their use for estimating human risk[1,2]. The problem stems from the expression of certain alleles which influence response to chemicals in various inbred strains, many of which were specifically selected to respond to particular chemicals or to have a high spontaneous incidence of tumours[3-6]. Thus, some toxicology testing protocols now involve laboratory outbred mice, on the rationale that such strains more closely resemble genetically heterogeneous human populations.

A key assumption in the use of outbred stocks is their genetic similarity to outbred mouse or human populations. Festing[7] has argued that the variation within an outbred stock is considerably smaller than that between inbred strains and has proposed the use of a panel of different inbred strains in a factorial design[7,8]. The true extent of gene variability in laboratory outbred stocks is unknown[8-10], and no data have been reported on stocks of the Swiss mouse, the most commonly used outbred mouse line.

We have applied the techniques and concepts of population genetics to the biochemical genetic characterisation of outbred Swiss mice from three major production colonies known to supply a large proportion of such mice for carcinogenesis and toxicological testing. The extent of gene–enzyme electrophoretic variation at 46 structural genes is presented in addition to the typing of several inbred Swiss strains. The results are analysed with respect to the following questions: (1) Do different isolated Swiss mouse colonies differ from each other in their population genetic structure? (2) Do outbred Swiss mice contain the same amount of variation observed in wild mouse populations, or has it been diminished by inbreeding, possibly through bottlenecks or by protection from the selective pressures of the wild state? (3) Is variation in Swiss mice qualitatively different (that is, with different variable loci, alleles or allele frequencies) from that in wild populations? In addition, the results of the analysis are related to considerations of population genetic theory of isolated populations.

History of the three Swiss mouse colonies

The original colony of Swiss mice in the US started from nine mice brought from Lausanne, Switzerland, in 1926 by Clara Lynch. Lynch[11] documented the genealogical history of the outbred strain starting from the Pasteur Institute in Paris until it reached the Rockefeller Institute where Webster maintained and dispersed Swiss and Swiss–Webster mice to researchers and commercial dealers. The three major colonies diverged from the Rockefeller colony in the late 1930s. The derivation of the outbred and inbred Swiss mouse strains is reconstructed in Fig. 1.

An estimate of gene–enzyme variation in Swiss mice

Between 40 and 50 6-week-old female non-sibling Swiss mice were obtained from each of the three colonies in addition to several mice from the Swiss inbred lines, from BALB/c and from C57BL/6. Extracts prepared from kidney, liver, red blood cells and plasma from each animal were subjected to gel electrophoresis and developed histochemically for 26 enzymes and haemoglobin. The 27 systems were selected from isozyme systems used in our laboratory for studies in somatic cell genetics[12-14]. The extent and character of allozyme (allelic isozyme) variation at each of 46 loci (Table 1) were determined by comparing the allozyme phenotype with the enzyme mobilities obtained from BALB/c or C57BL/6 mice standards run in the same gel[3,15-17]. The utility of these systems in somatic cell genetics does not depend on allozyme polymorphism, and thus minimises the bias of published systems which are mostly polymorphic[18]. Of the 46 loci, 38 have been mapped to specific chromosomal sites in the mouse (Table 1), indicating that they are genetically autonomous of each other.

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* To whom reprint requests should be addressed.

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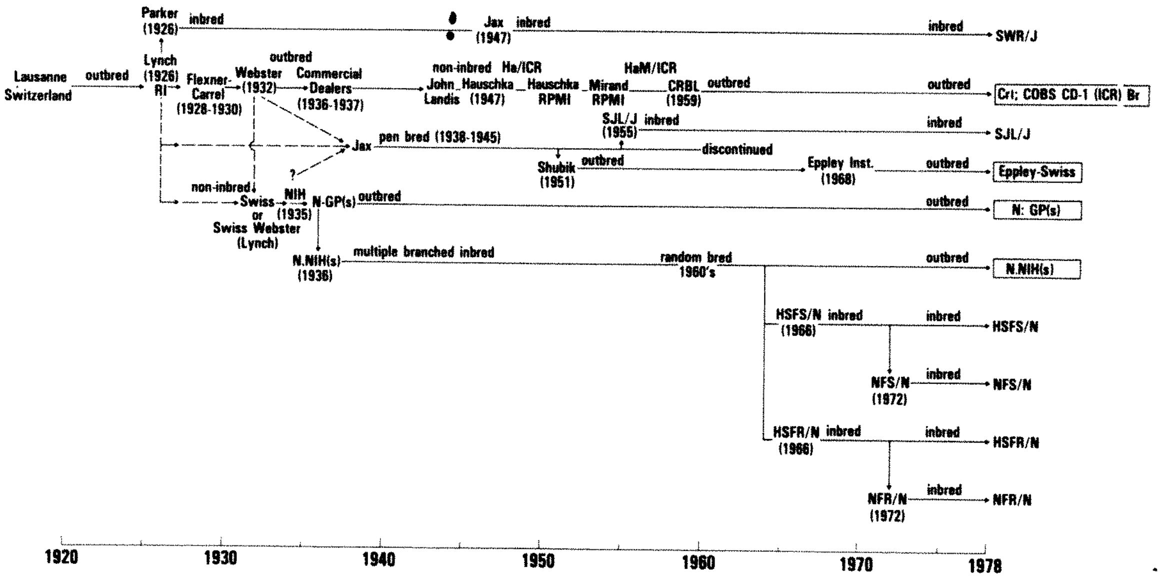

Fig. 1 Genealogical history of Swiss outbred mice and inbred derivatives. **CD1 colony:** In 1947, Hauschka established the Ha/ICR outbred stock from 100 female and 30 male 'Swiss–Webster' mice obtained from John Landis in Hagerstown, Maryland[47]. The origin of Landis' stock is obscure. When Hauschka moved from the Institute of Cancer Research, Philadelphia, to Roswell Park Memorial Institute, he passed some of the mice to E. A. Mirand. In 1957, Mirand sent 50 female mice to Charles River Breeding Laboratories where they founded the Cr1 : COBS.CD-1(ICR)BR colony (G. Pucak, personal communication). The CD-1 stock is now bred on a 1 × 1 system adopting the 'Poiley method', a systematic circular method used to maximise outbreeding. A protected nucleus colony of 300–400 breeders is kept in sterile plastic film isolators and several production colonies produce 10,000–12,000 CD-1 pups each per week. To maintain homogeneity, pups from different production colonies are added to the nucleus periodically. **Eppley–Swiss colony**: The Jackson Laboratory, Bar Harbor, received Swiss and Swiss–Webster outbred mice from three sources (C. Lynch, L. Webster and one unknown) between 1938 and 1945 (ref. 3) (J. Staats, J. Dorey, personal communications). These outbred lines were pen bred until 1955. In 1951, P. Shubik, Chicago Medical School, obtained Swiss mice from the pen-bred colony (B. Toth, personal communication) and started a closed, outbred colony. Shubik moved the colony to the Eppley Institute, Omaha, Nebraska, in 1968, where it is now bred on a 1 × 1 scheme, using an extensive labelling system to insure random breeding in an isolated breeder colony of 150–180 pairs. The Eppley–Swiss colony produces 18,000–26,000 mice per yr. **N.NIH(s) colony**: In 1935 the NIH received an undetermined number of outbred Swiss mice from the Rockefeller Institute that were the founders of the general purpose colony N : GP(s). In 1936, the N.NIH(s) was derived from the N : GP(s) colony. The N.NIH(s) colony was bred from 1936 to the 1960s by a multiple-branched inbreeding system described as a "brother–sister mating without following any systematic plan" (ref. 40). In the early 1960s a strict random mating scheme was resumed for the N.NIH(s) stock (C. T. Hansen, personal communication). The N.NIH(s) stock was rederived in 1972 by hysterectomy and foster nursing of mice to establish a specific pathogen-free (SPF) colony[48]. This nucleus colony consists of 120 pairs, is bred by a circular pair mating system and is selected for high productivity (C. T. Hansen, personal communication). The N.NIH(s) colony produces 140,000 mice per yr and the N : GP(s) colony 110,000 per yr. **Inbred strains**: In 1926, an inbred derivative of Lynch's Swiss strain, SWR, was established by Raymond Parker at the University of Toronto. In 1947, the SWR line was supplied to the Jackson Laboratory which continued the inbred line as SWR/J. The SJL/J strain was derived from the pen-bred Swiss strain at the Jackson Laboratory around 1955 when the outbred line was discontinued. In 1966, two inbred lines were derived from the N.NIH(s) colony. These lines were selected for their reaction to histamine after treatment with *Bordetella pertussis* and were thereby named HSFR/N (histamine-sensitive-factor resistant) and HSFS/N (histamine-sensitive-factor sensitive). In 1972, two new inbred lines, NFS/N and NFR/N, were rederived from HSFS/N and HSFR/N, respectively. During derivations of the present colonies, there were various periods of bottlenecks of unconfirmed size as well as periodic artificial selection of each outbred stock for fecundity (refs 11, 40, 47 and G. Pucak, J. Staats, J. Dorey, C. T. Hansen, personal communications). The following abbreviations were used: Jax: the Jackson Laboratories, Bar Harbor, Maine; RPMI: Roswell Park Memorial Institute, Buffalo, New York; NIH: NIH, Bethesda, Maryland; CRBL: Charles River Breeding Labs, Wilmington, Massachusetts; RI: Rockefeller Institute, New York, New York.

The genetic constitution of the three outbred mouse populations and six inbred derivative strains is presented for each locus in Table 1. Of the 46 loci tested, 37 (80.4%) were monomorphic in each population and, interestingly, for the same allele in all three colonies. Conversely, 9 of the 46 loci (19.6%) were polymorphic for at least two alleles in one or more of the three outbred colonies. Alternative alleles were found to be homozygous in different inbred strains at each of the polymorphic loci except *Gpt-1*. In addition, two of the loci which were invariant within the outbred colonies (*Es-3* and *Es-6*) were fixed for different alleles in derivative inbred strains. The finding of differential fixation for each polymorphic allele in different inbred derivatives is taken as evidence that the observed variation has a genetic basis, and that the present polymorphisms probably existed in and are thus derived from Lynch's original colony.

The allelic frequencies of each of the nine polymorphic loci in the three outbred mouse colonies are presented in Table 2. Six of the nine loci are polymorphic in all three populations, and three loci, *Gpi-1*, *Id-1* and *Got-2*, have become monomorphic or nearly so in at least one of the three colonies. The allelic frequencies were used to estimate the expected frequency of heterozygotes ($2pq$) for each population at each polymorphic locus[18–20] also presented in Table 2. Each locus was tested for conformity to the Hardy–Weinberg equilibrium (HWE) by a χ^2 test with one degree of freedom. Two departures from expectation for a random breeding population were observed: *Hbb* ($\chi^2 = 16.45$, $P \leq 0.0005$) and *Id-1* ($\chi^2 = 7.6$, $0.005 \leq P \leq 0.01$), both in the Eppley population. Similar distortions of *Hbb* genotypic frequencies have been reported in several wild mouse populations[21–24]. The conformity of 19 of 20 tested loci (excluding *Hbb*) to HWE effectively excludes any marked influence of assortive mating, heterosis, migration or selection on the operation of random mating in these populations. As HWE is a stable one (it depends on allele frequencies at the time of sampling and is independent of allele frequencies in previous generations), the sample frequencies give little indication of the randomness (or otherwise) of mating in the 50-yr history of Swiss strains.

Comparison of Swiss colonies to wild mouse populations

The proportion of polymorphic loci measured in the Swiss colonies and a number of feral mouse populations is presented in Table 3. Also presented for each population is the average heterozygosity, which is a better estimate of genic variation in random breeding populations because it takes gene frequencies into account. The Eppley colony is the most polymorphic of the Swiss mice, with 19.6% of its loci polymorphic, compared with 17.4% CD-1, and 13.4% N.NIH(s). The average, 16.8%, is not quite as high as the average calculated for the natural mouse populations listed in Table 3, including two California populations (Lake Casitas and Bouquet Canyon) which were tested for the same 46 loci[24]. The average heterozygosity of Swiss mice is 0.071, which is only slightly lower than the average heterozygosity calculated for the two California feral mouse populations tested for the same 46 loci (0.084) and previously published estimates for mice (Table 3). The values are also comparable to variability estimates of human populations reported by Harris and Hopkinson (0.067)[25] and Nei and Roychoudhury (0.10)[26].

The similarity between the three Swiss colonies and feral mice tested in this laboratory was quantitated using Nei's genetic distance[27,28] (Table 4). This statistic measures the degree of allelic substitution between populations based on electrophoretic mobility of several gene–enzyme systems. Within the limits of Nei's assumptions[27], the genetic distance estimates increase proportionately with the amount of time the populations have been reproductively isolated. The average distance between the three Swiss colonies is slight (0.023), approximately the same as the distance between the Lake Casitas and Bouquet Canyon (0.019) which are local populations separated by 40 miles in southern California[24]. The genetic distance between the Swiss mice and the California mice (average 0.062) is three times the distance within the two groups, indicating an isolation of Swiss from American mice three times longer than isolation within either group. This observation is consistent with the European origin of Swiss mice some 70 years ago.

Table 1 Allozyme composition of Swiss outbred mice populations and derivative inbred mouse strains

	Outbred			Inbred derivatives					
Gene symbol	N.NIH(s)	CD-1	Eppley–Swiss	SWR/J	SJL/J	NFS	NFR	HSFS	HSFR
Acp-1 (12)	A	A	A	—	—	A	A	A	A
Ada	A	A	A	A	A	A	A	A	A
Aprt (8)	A	A	A	A	A	A	A	A	A
Ak-1 (2)	A	A	A	—	—	A	A	A	A
Ak-2 (4)	A	A	A	—	—	A	A	A	A
Adh-2	A	A	A	—	—	A	A	A	A
Es-1 (8)	b	b	b	b	b	b	b	b	b
Es-2 (8)	b	b	b	b	b	b	b	b	b
Es-3 (11)	c	c	c	c	c	a	c	c	c
Es-5 (8)	b	b	b	—	—	b	b	b	b
Es-6 (8)	a	a	a	b	b	a	a	a	a
Es-10 (14)	a	a	a	a	a	a	a	a	a
Es-s	A	A	A	—	—	A	A	A	A
G6PD (X)	A	A	A	—	—	A	A	A	A
Gpi-1 (7)	b	a, b	a, b	b	a	b	b	b	b
Got-1 (19)	a	a	a	a	—	a	a	a	a
Got-2 (8)	a, b	a, b	a, b	a	b	a	a	a	a
Gpt-1 (15)	a, c	a, c	a, c	a	a	a	a	a	a
Gapdh	A	A	A	—	—	A	A	A	A
Gr-1 (8)	a, b	a, b	a, b	b	b	b	b	a	b
Glo-1 (17)	a, b	a, b	a, b	—	a	a	a	a	b
Hbα (11)	A	A	A	A	A	A	A	A	A
Hbb (7)	s, d	s, d	s, d	s	s	d	d	d	d
Hk-1 (10)	A	A	A	—	—	A	A	A	A
Hprt (X)	A	A	A	—	—	A	A	A	A
Id-1 (1)	a, b	a	a, b	a	b	a	a	a	a
Id-2 (7)	A	A	A	—	—	A	A	A	A
Ldh-1 (7)	A	A	A	A	A	A	A	A	A
Ldh-2	A	A	A	—	—	A	A	A	A
Mor-1 (5)	a	a	a	—	—	a	a	a	a
Mor-2	A	A	A	A	A	A	A	A	A
Mod-2 (7)	b	b	b	b	b	b	b	b	b
Mod-1 (9)	a, b	a, b	a, b	a	a	a	a	a	b
Mpi-1 (9)	b	b	b	b	b	b	b	b	b
Np-1 (14)	a	a	a	a	a	a	a	a	a
Pep-1 (18)	A	A	A	A	A	A	A	A	A
Pep-2 (10)	A	A	A	—	—	A	A	A	A
Pep-3 (1)	b	b	b	b	b	b	b	b	b
Pep-4 (7)	A	A	A	—	—	A	A	A	A
Pep-7 (5)	A	A	A	—	—	A	A	A	A
Pgd-1 (4)	b	b	b	b	b	b	b	b	b
Pgm-1 (5)	a, b	a, b	a, b	b	b	a	b	a	a
Pgm-2 (4)	a	a	a	a	a	a	a	a	a
Pyp (10)	A	A	A	—	—	A	A	A	A
Sod-1	A	A	A	—	—	A	A	A	A
Sod-2	A	A	A	—	—	A	A	A	A

The following enzymes were scored from mouse liver extracts: acid phosphatase (*Acp-1*), adenosine deaminase (*Ada*), glucose-6 phosphate dehydrogenase (*G6PD*); from red blood cell lysates: esterase-3 (*Es-3*), esterase-10 (*Es-10*), glucose phosphate isomerase (*Gpi-1*), haemoglobin α, β chain (*Hbα*, *Hbb*), phosphoglucomutase-1 and -2 (*Pgm-1* and *Pgm-2*); from serum: esterase-5 (*Es-5*), esterase-s (*Es-s*); from kidney: adenine phosphoribosyl transferase (*Aprt*), adenylate kinase-1 (*Ak-1*), adenylate kinase-2 (*Ak-2*), alcohol dehydrogenase (*Adh*), esterase-1 (*Es-1*), esterase-2 (*Es-2*), esterase-6 (*Es-6*), glutamate oxaloacetate transaminase(soluble) (*Got-1*), glutamate oxaloacetate transaminase(mitochondrial) (*Got-2*), glutamate pyruvate transaminase(soluble) (*Gpt-1*), glyceraldehyde 3-phosphate dehydrogenase (*Gapdh*), glutathione reductase (*Gr-1*), glyoxylase (*Glo-1*), hexokinase (*Hk-1*), hypoxanthine phosphoribosyl transferase (*Hprt*), isocitrate dehydrogenase(soluble) (*Id-1*), isocitrate dehydrogenase(mitochondrial) (*Id-2*), lactate dehydrogenase-A (*Ldh-1*), lactate dehydrogenase-B (*Ldh-2*), malate dehydrogenase(mitochondrial) (*Mor-1*), malate dehydrogenase(soluble) (*Mor-2*), malic enzyme(mitochondrial) (*Mod-2*), malic enzyme(soluble) (*Mod-1*), mannose phosphate isomerase (*Mpi-1*), purine nucleoside phosphorylase (*Np-1*), peptidase-1 (*Pep-1*), peptidase-2 (*Pep-2*), peptidase-3 (*Pep-3*), peptidase-4 (*Pep-4*), peptidase-7 (*Pep-7*), 6-phosphogluconate dehydrogenase (*Pgd-1*), pyrophosphatase (*Pyp*), superoxide dismutase(soluble) (*Sod-1*), superoxide dismutase(mitochondrial) (*Sod-2*). The vertical starch gel electrophoresis apparatus (Buchler) was used in combination with 12% Electrostarch (Electrostarch) for each system except adenosine and hypoxanthine–guanine–phosphoribosyl transferase. These systems were assayed following electrophoresis in 7% acrylamide in an EC-Apparatus (E.C. Apparatus). Phosphoribosyl transferases were detected by incubation of the gels with ^{14}C-labelled substrates. The phosphorylated products were precipitated with $LaCl_3$, and the gels were dried and exposed to autoradiography[41]. Haemoglobin was separated in gels containing 0.8% cystamine dihydrochloride[42]. The remaining enzymes examined were separated and visualised by modifications of standard isozyme procedures[43–45]. A = no name assigned to this allele. Numbers in parentheses after gene symbol are chromosome assignments.

Table 2 Polymorphic loci of Swiss mice

Enzyme (chromosome)	N.NIH(s)			CD-1			Eppley–Swiss		
	Allele frequency	$2pq$	HWE χ^2	Allele frequency	$2pq$	HWE χ^2	Allele frequency	$2pq$	HWE χ^2
Gpi-1 (7)	$b=1.0$	—	—	$a=0.43$ $b=0.57$	0.49	2.1	$b=0.40$ $a=0.60$	0.48	1.11
Gr-1 (8)	$a=0.35$ $b=0.65$	0.46	0.85	$a=0.56$ $b=0.44$	0.49	1.8	$a=0.47$ $b=0.53$	0.50	0.31
Got-2 (8)	$b=0.97$ $a=0.03$	0.06	—	$b=0.60$ $a=0.40$	0.48	0.39	$b=0.27$ $a=0.73$	0.39	2.76
Gpt-1 (15)	$a=0.67$ $b=0.33$	0.44	—	$a=0.80$ $b=0.20$	0.32	—	$a=0.57$ $b=0.43$	0.49	—
Glo-1 (17)	$a=0.64$ $b=0.36$	0.46	0.38	$a=0.86$ $b=0.14$	0.24	2.36	$a=0.62$ $b=0.38$	0.47	0.24
Hbb (7)	$s=0.56$ $d=0.44$	0.49	0.64	$s=0.82$ $d=0.18$	0.30	1.86	$s=0.40$ $d=0.60$	0.48	$\boxed{16.45}$
Id-1 (1)	$a=0.97$ $b=0.03$	0.06	—	$a=1.0$	—	—	$a=0.70$ $b=0.30$	0.42	$\boxed{7.6}$
Mod-1 (9)	$b=0.12$ $a=0.88$	0.21	0.70	$b=0.83$ $a=0.17$	0.28	0.74	$b=0.27$ $a=0.73$	0.39	2.17
Pgm-1 (5)	$a=0.37$ $b=0.63$	0.47	3.85	$a=0.57$ $b=0.43$	0.49	1.45	$a=0.64$ $b=0.36$	0.46	2.69

$2pq$ is the predicted frequency of heterozygotes based on allele frequencies. HWE χ^2 is the value obtained in testing conformity of observed phenotype frequencies to those expected in conditions of Hardy–Weinburg equilibrium.

The Swiss outbred mouse polymorphisms are not qualitatively different from those reported for wild mouse populations[29–32]. Eight of the nine polymorphic loci (excluding *Glo-1*)[33] seen in Swiss outbred mice vary between inbred strains[15–17] and in wild mouse surveys. Of the 37 gene–enzyme systems which are monomorphic in Swiss outbred mice, 9 (*Es-1, Es-2, Es-3, Es-5, Mor-1, Pep-2, Pgd-1, Pgm-2* and *Sod-1*) were polymorphic in at least one natural population previously studied, but only 3 (*Es-2, Es-3, Pep-2*) were polymorphic in the majority of the natural populations sampled[23,29–32]. The other gene enzyme systems were either monomorphic or never tested in natural populations.

Have the Swiss colonies been supplemented by non-Swiss mice?

It is, unfortunately, impossible to determine whether or not any of the Swiss stocks has been contaminated with non-Swiss mice during their history. Our data do, however, strongly suggest that migration from non-Swiss mice significant enough to change the genetic constitution of the stock has not occurred in any of the three colonies. First, the genetic distance (Table 4) between the colonies is small and approaches the limits of population identity provided by this statistic[27,28]. The inter-colony distances are lower than 140, distances calculated between 18 different feral mice populations previously studied (S. J. O'Brien, in preparation). Second, two of the loci which were polymorphic in Swiss mice, *Gpi-1* and *Pgm-1*, are monomorphic in American populations[24,29], where the colonies developed, and polymorphic in European mice, the purported founders of Swiss mice. Third, *Glo-1* is monomorphic in wild mice[24], and between all inbred strains (excluding HSFR and MA/MyJ, Swiss derivatives[33]), but is polymorphic at appreciable frequencies in each of the Swiss colonies. Fourth, the Swiss inbred strains (Table 1) are fixed for each of the different alleles at each locus polymorphic in the outbred colonies. These genetic distributions are characteristic of each colony and thereby emphasise their consanguinity with each other and their distinctiveness from other mouse populations which might have been a source of migrating genes.

Swiss mice as an island population

The Swiss mouse has been isolated from its natural environment for over 50 yr (approximately 175 generations) and the colonies studied have been separated for over 30 yr (~ 122 generations). If, as claimed by their keepers, they have not been supplemented by non-Swiss mice during this period, they can be considered as characteristic island populations[34], isolated from migration and the selective rigours of the wild state. The retention of considerable genic variability comparable to that of mainland populations has been observed in feral mice in Hawaii[21] and Skokholm Island off the British mainland[31,32], and in six island populations of *Drosophila willistoni* in the West Indies[35]. In addition, at least one laboratory population of *Drosophila melanogaster* retained levels of polymorphism nearly identical to those of wild populations after laboratory propagation for over 20 yr (520 generations)[36].

Table 3 Proportion of loci estimated to be polymorphic and the proportion of the genome estimated to be heterozygous in mouse populations

Populations	No. of loci	No. of populations	Proportion of polymorphic loci (%)	Average heterozygosity	Refs
Swiss laboratory mice					
N.NIH(s)	46	1	13.4	0.056	This study
CD-1	46	1	17.4	0.067	This study
Eppley–Swiss	46	1	19.6	0.089	This study
Average	46	3	16.8	0.071	This study
Wild feral mice					
Lake Casitas, Calif. *Mus musculus domesticus*	46	1	24	0.099	24
Bouquet Canyon, Calif. *Mus musculus domesticus*	46	1	17	0.077	24
Hallowell Farm, Calif. *Mus musculus brevirostri*	40	10	30	0.11	29
Denmark *Mus musculus musculus*	41	4	29	0.087	30
Denmark *Mus musculus domesticus*	41	2	22	0.070	30
British mainland	22	6	29	0.065	31
British Islands	22	20	17	0.045	31
Balenya, Spain *Mus hispanicus* (*spretus*)	56	1	20	0.034	46

The slight loss in genetic variance we observe here compared with wild mice or previous variability in Swiss populations indicated by variant inbred mice at two additional (to presently polymorphic) loci is probably due to genetic drift. The following calculation tends to support this notion. To estimate the rate of lost variation due to drift in isolated populations, Fisher[37] and Wallace[38] derived the equation

$$H_t = H_0 e^{-t/2N}$$

where H_t is the proportion of heterozygotes at generation t, H_0 is the proportion of heterozygotes at generation 0, and N is the effective breeding size of the population. We can estimate the minimum average breeding size (N) of the three colonies which would result in the observed average heterozygosity by estimating H_0 from data for either inbred mice or wild mice as a norm over 175 (+) generations in the laboratory. If $H_0 = 0.12$ (assuming 11/46 heterozygous loci originally, at a maximum heterozygosity of 0.5 at each locus), then $N = 118$ for N.NIH(s), 154 for CD-1 and 293 for Eppley. If we use $H_0 = 0.10$ (from the highest mouse population, Lake Casitas, in Table 3), the N values become 138 for NIH(s), 201 for CD-1 and 665 for Eppley. Thus, one would expect the average heterozygosity seen in the Swiss mice from genetic drift alone if their breeding sizes approximated the calculated values. With smaller breeding sizes the genetic variance would be less than we observe. The actual breeding sizes of the colonies are clearly well within these predicted ranges. In fact, one generation bottlenecks (for example, Lynch's original transport or caesarian rederivations of Swiss mice) of as few as 10 mice would have resulted in the loss of less than 10% of the included variation[39]. From these considerations, the possibility of inbreeding (conscious or not) to the extent of significantly diminishing the genetic variance of Swiss mice is unlikely.

Conclusions

The results of this survey of three isolated colonies of Swiss mice indicate that a considerable amount of genetic variation persists in the stock. The average heterozygosity of Swiss mice (0.07) is very similar to estimates for feral mouse[24,29–32] and human[25,26] populations. Sampling of six inbred mouse strains derived at different periods in the history of Swiss mice provided the equivalent of fixed gametic samples from the generation of origin of the inbred line. The inbred samplings reinforce the age and persistence of the presently observed polymorphisms and indicate the existence of variability at two loci now monomorphic in Swiss mice. The population structure of the three colonies today is consistent with prior fixation at these loci due to genetic drift, possibly during population bottlenecks or short periods of inbreeding[11,40]. The populations today appear to be functionally random bred insofar as their genotype frequencies distribute as a bionomal Hardy–Weinberg equilibrium.

With respect to the three questions posed at the outset, the data are quite specific: (1) The three Swiss colonies are quite similar to each other in the extent and character of their included genic variation. The genetic distance between colonies is low, as would be predicted of populations separated for a few hundred generations. (2) The Swiss mice contain slightly less variation than do wild mouse populations, but the similarities to feral populations are more apparent than the differences. (3) The alleles observed, both monomorphic and polymorphic, are for the most part the same ones found in wild populations.

With reference to the question of efficacy of using outbred mice in carcinogenesis and toxicology protocols, the data presented here are precise, although specifically limited. The Swiss mouse colonies do seem to provide a realistic model of isolated outbred mouse populations in terms of persistence of genetic variation. If one chooses to test outbred animals, these colonies will suffice. Thus, the argument that random-bred laboratory strains are probably inbred seems to be incorrect. Nevertheless, the decision to use outbred as opposed to inbred animals for testing protocols must ultimately be made on the basis of the information desired from the individual test, considerations which are beyond the scope of this report.

Table 4 Genetic distance (D) and identity (I) estimates of Swiss and wild mouse populations

	I				
D	Lake Casitas	Bouquet Canyon	N.NIH(s)	CD$_1$	Eppley
Lake Casitas		0.981	0.928	0.939	0.969
Bouquet Canyon	0.019		0.922	0.931	0.949
N.NIH(s)	0.075	0.081		0.976	0.975
CD$_1$	0.063	0.071	0.025		0.980
Eppley	0.032	0.052	0.025	0.020	

I equals the probability of allelic identity of any randomly selected genes at any locus in each of two test populations. D equals the average number of gene differences per locus between individuals from the two test populations. Algebraically

$$I = J_{xy}/\sqrt{J_x J_y}$$

$$D = -\ln I$$

where J_{xy} is the arithmetic mean of $j_{xy} = \sum_i x_i y_i$ over all loci, J_x is the arithmetic mean of $j_x = \sum_i x_i^2$ over all loci, and x_i (or y_i) is the frequency of the ith allele in the first (or second) population[28].

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Transglutaminase is essential in receptor-mediated endocytosis of α_2-macroglobulin and polypeptide hormones

Peter J. A. Davies, Dana R. Davies, Alexander Levitzki*, Frederick R. Maxfield, Pierre Milhaud, Mark C. Willingham & Ira H. Pastan

Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205

The receptor-mediated endocytosis of α_2-macroglobulin can be inhibited by a diverse group of chemical compounds all of which share the property of being inhibitors of one form of cellular transglutaminase. The present results strongly suggest that protein cross-linking may be essential for receptor-mediated endocytosis of some protein and polypeptide hormones.

RECEPTOR-mediated endocytosis is the process by which cells bind and internalise specific extracellular ligands. The process is responsible for the uptake of a variety of proteins and peptides, including the serum proteins low density lipoprotein (LDL)[1] and α_2-macroglobulin (α_2M)[2–5], several lysosomal hydrolases[6,7] and the polypeptide hormones insulin and epidermal growth factor (EGF)[8–12]. For several of the ligands, ultrastructural studies have shown that internalisation occurs over clathrin-coated regions of the membrane[1,4,11,12]. The internalisation of α_2M follows the same pathway as the internalisation of insulin and EGF[2]; unoccupied receptors are diffusely distributed on the cell surface of cultured fibroblasts and cluster over clathrin-coated pits after the ligand binds[2,8,10]. LDL receptors, on the other hand, seem to be concentrated over coated regions even in the absence of ligand binding[1]. These coated regions pinch off to form endocytotic vesicles which transfer the internalised ligands to other organelles.

Although the steps involved in receptor-mediated endocytosis have been described, the molecular mechanisms involved in the process are unknown. To understand the mechanism of ligand internalisation we have searched for inhibitors of receptor-mediated endocytosis. Recently, we have observed that the first step in the process, the aggregation of ligand–receptor complexes over coated pits, required extracellular Ca^{2+} and was inhibited by primary alkylamines[13]. This observation suggested several potential mechanisms of inhibition, including the possibility that a transglutaminase might be important in the process. Transglutaminase is an intracellular enzyme found in many types of cells. Some transglutaminases covalently cross-link proteins by forming ε-(γ-glutaminyl)-lysine cross-bridges (for review see ref. 14). These enzymes require Ca^{2+} for activity and can be inhibited by a variety of primary amines. Several forms of transglutaminase are known. Guinea pig liver transglutaminase and plasma transglutaminase have been purified, and a wide variety of specific inhibitors of these enzymes have been identified[14–16].

To test the hypothesis that a transglutaminase is involved in receptor-mediated endocytosis, we tested a wide variety of inhibitors of transglutaminases to see if they blocked ligand clustering and internalisation. We have found an excellent correlation between the ability of compounds to inhibit a

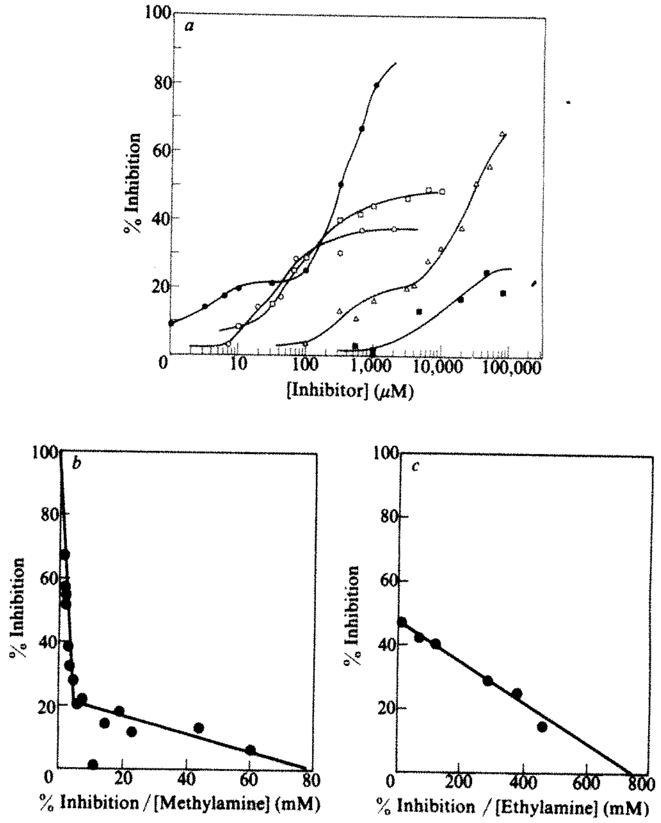

Fig. 1 Inhibition of transglutaminase in CHO extracts. *a*, log dose–response curves for inhibition of CHO transglutaminase by dansylcadaverine (●), ethylamine (□), bacitracin (○), methylamine (△) and tolbutamide (■). *b*, Plot of fractional inhibition against fractional inhibition/inhibitor concentration (Eadie–Hofstee) for methylamine on CHO transglutaminase. *c*, As (*b*) but for ethylamine. CHO cells were grown in suspension culture in Dulbecco's modified essential media (DMEM) containing 10% heated calf serum to a density of 1 × 10^6 cells per ml. Cells were pelleted, washed twice in phosphate-buffered normal saline (PBS), resuspended to 1–4 mg cell protein per ml in PBS and lysed by sonication. Extracts were frozen at −70 °C and then thawed and assayed for transglutaminase activity. Assays measured the Ca^{2+}-dependent incorporation of ^{3}H-putrescine into casein using a filter paper assay[18]. The assay mixture (200 μl) contained 20 mM Tris-HCl, *p*H 7.4, 2 mg ml^{-1} casein (Mann), 5 mM mercaptoethanol, 5 mM ^{3}H-putrescine (40 c.p.m. pmol^{-1} NEN) and various concentrations of inhibitor. All inhibitors were adjusted to *p*H 7.4 before addition to the assay. The reaction was initiated by addition of ^{3}H-putrescine and incubation at 37 °C and duplicate aliquots (25 μl) were taken at 3 and 6 min, spotted on filter papers (Whatman 3MM) and precipitated by addition to cold 10% trichloroacetic acid. After washing, dehydrating and drying[18], filters were counted in a toluene-based scintillation cocktail (Econofluor, NEN) at an efficiency of 13%. The background activity measured in the absence of Ca^{2+} (5 mM EGTA) was 50–150 c.p.m. and was subtracted from all values. Enzyme activity varied over 4–10 nmol putrescine incorporated per min per mg cell protein, depending on the preparation used. Data shown represent means of duplicate determinations pooled from at least two separate experiments.

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* Permanent address: The Hebrew University, Jerusalem, Israel.

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fibroblast transglutaminase and their ability to inhibit the aggregation and internalisation of α_2M. It therefore seems possible that protein cross-linking is essential for the endocytosis of hormones and α_2-macroglobulin.

Inhibition of fibroblast transglutaminase

Transglutaminase activity in fibroblasts was assayed by measuring Ca^{2+}-dependent covalent incorporation of ^{3}H-putrescine into casein[17,18]. Transglutaminase activity (4–10 nmol per min per mg cell protein) was 10–20 times higher in extracts of CHO cells than in normal rat kidney (NRK) fibroblasts or BALB 3T3 fibroblasts. Therefore, we used CHO extracts to characterise inhibitors of fibroblast transglutaminase.

We first examined methylamine as an inhibitor of CHO transglutaminase. As shown in Fig. 1*a*, low concentrations of methylamine produced partial inhibition of CHO transglutaminase activity but much higher concentrations were required to produce complete inhibition. Kinetic analysis of the inhibition curve (Fig. 1*b*) showed that the inhibition consisted of two components. A high-affinity component (K_i 0.2 mM) accounted for 25% of the total activity, and a low-affinity component (K_i 20 mM) accounted for the remainder. The fraction of high-affinity component varied from 25 to 50% total activity depending on the preparation of cells used. Similar results were obtained with other fibroblast cell lines, NRK and BALB 3T3, but because of the low specific activity and a lower fraction of the high-affinity transglutaminase pool, quantitative measurements were difficult.

Ethylamine was more potent than methylamine (Fig. 1*a*), but even high concentrations produced only partial inhibition of transglutaminase activity. Kinetic analysis of the ethylamine inhibition curve (Fig. 1*c*) showed a straight line, indicating a single component of inhibition, with K_i 35 μM, that accounted for 45% of the total enzyme activity. *n*-Propylamine, isopropylamine and *n*-hexylamine were all equivalent to ethylamine as inhibitors of CHO transglutaminase (data not shown).

Dansylcadaverine, a very potent inhibitor of liver and serum transglutaminases[16,19], is also a potent inhibitor of CHO transglutaminase (Fig. 1*a*). Concentrations of 1–100 μM produced partial inhibition of the enzyme activity whereas higher concentrations produced complete inhibition. As with methylamine, kinetic analysis demonstrated two components of inhibition, a high-affinity component (K_i 2.5 μM, 28% total activity) and a low-affinity component (K_i 200 μM, 72% of total activity).

We have also identified two novel inhibitors of CHO transglutaminase activity, the peptide antibiotic, bacitracin, which blocks hormone degradation[20] and the sulphonylurea, tolbutamide. As shown in Fig. 1*a*, both produced partial inhibition of CHO transglutaminase; bacitracin had a K_i comparable to that of ethylamine, 35 μM. Tolbutamide was much less active, the K_i being approximately 2 mM.

Dansylcadaverine inhibition of receptor-mediated endocytosis in CHO cells

α_2-Macroglobulin binds to saturable cell-surface receptors[5]. As shown in Table 1, rhodamine-labelled α_2M (R-α_2M) retains the ability to bind to these receptors. The bound α_2M becomes concentrated over clathrin-coated pits[4] and is internalised in endocytotic vesicles[21].

As the biochemical studies on transglutaminase were carried out in CHO cell extracts, we also characterised the clustering and internalisation of α_2M by these cells. The experimental protocol was similar to that reported by Maxfield *et al.*[13]. CHO cells in serum-free culture media were exposed to R-α_2M for 30 min at 37 °C. Unbound R-α_2M was then removed and the cells were washed and fixed with formaldehyde. The bound R-α_2M was viewed in an epifluorescence microscope using a silicon intensifier television camera[22]. Data presented are photographs of a video monitor.

Figure 2 shows receptor-mediated endocytosis of R-α_2M by CHO cells and the effect of dansylcadaverine on the process. Figure 2*a* shows the punctuate pattern of fluorescence which is seen after a 30-min incubation with R-α_2M at 37 °C. This pattern represents the result of the clustering and internalisation of the R-α_2M. The dashed lines denote the margins of individual cells.

Table 1 Fluorescence intensities of NRK cells incubated with rhodamine-α_2-macroglobulin

Incubation conditions	Fluorescence intensity (arbitrary units)
R-α_2M (10 μg ml^{-1})	4±2
R-α_2M (30 μg ml^{-1})	18±6
R-α_2M (100 μg ml^{-1})	57±16
R-α_2M (300 μg ml^{-1})	95±20
R-α_2M(100 μg ml^{-1})+α_2M (2 mg ml^{-1})*	9±4
Dansylcadaverine (50 μM), 30 min; R-α_2M (100 μg ml^{-1})†	43±12
SFM, 30 min; R-α_2M (100 μg ml^{-1})†	48±15

NRK cells were plated on Falcon tissue culture dishes and grown to confluency. The cells were rinsed with serum-free medium (SFM) and incubated with the indicated concentrations of R-α_2M in SFM for 30 min at 37 °C. Cells were rinsed, fixed and observed as described in Fig. 2 legend. Clusters were observed at all concentrations of R-α_2M tested. For intensity measurements, an EMI 9658 R photomultiplier tube was mounted on the microscope, and the incident illumination was passed through a monochromator (Instruments SA, model H10) with a spectral bandpass of 8 nm in addition to the narrow bandpass filter on the microscope. The diameter of the illumination beam was approximately 30 μm in the focal plane. The values shown are the mean and standard deviation of the intensities at 20 randomly selected locations on the dish. Untreated cells had an intensity of 5 units and this was subtracted from all the values shown. Duplicate dishes gave the same average value ±10%. Similar results were obtained with CHO cells. Trypsin treatment of the R-α_2M to form trypsin-α_2M complexes[5] did not significantly alter the binding.

* α_2M was added to the cultures 5 min before R-α_2M.

† Dansylcadaverine was added 30 min before R-α_2M; control dishes were incubated with SFM.

As can be seen in Fig. 2*b*, cells treated with 20 μM dansylcadaverine show a marked reduction in the number and intensity of fluorescent spots. There is a compensating increase in diffusely distributed fluorescence. The effect is more marked with 100 μM dansylcadaverine (Fig. 2*c*). Few clusters are seen and R-α_2M seems to be diffusely distributed on the cell surface. By titration it is possible to estimate roughly the concentration of inhibitor required to produce a 50% decrease in the number and intensity of fluorescent spots (ID_{50}). Concentrations three- to fivefold below the ID_{50} produced no observable effect. Concentrations near the ID_{50} partially inhibited clustering (Fig. 2*b*), and concentrations three- to fivefold higher than the ID_{50} almost completely blocked clustering (Fig. 2*c*). In the case of dansylcadaverine, we estimated an ID_{50} of 20 μM. The effect of dansylcadaverine was not unique to CHO cells. Equivalent concentrations of dansylcadaverine blocked clustering and internalisation of R-α_2M in NRK cells and Swiss 3T3 fibroblasts (data not shown). Dansylcadaverine was also equally effective in inhibiting the clustering of rhodamine-conjugated EGF on NRK cells (Fig. 2*e, f*) and on Swiss 3T3 cells (data not shown).

To rule out an effect of dansylcadaverine on R-α_2M binding, CHO cells were incubated for 30 min with dansylcadaverine and then for 15 min more with added R-α_2M. Both unbound R-α_2M and dansylcadaverine were then washed out and the fate of the diffusely bound R-α_2M was followed (Fig. 2*d*). Within 30 min, bright fluorescent spots formed, indicating that ligand previously bound to receptor was competent to cluster and be internalised. Dansylcadaverine did not significantly affect the fluorescent intensity of cells incubated with R-α_2M (Table 1). Furthermore, dansylcadaverine did not reduce the binding of ^{125}I-EGF by cells (H. Haigler and I.H.P., in preparation).

Correlation of inhibition of clustering with inhibition of transglutaminase

Having established conditions for assaying inhibition of transglutaminase and also inhibition of receptor-mediated endocytosis, we compared the potency of various compounds as inhibitors of the two processes. The results are plotted in Fig. 3. The data obtained with CHO cells are shown by solid circles;

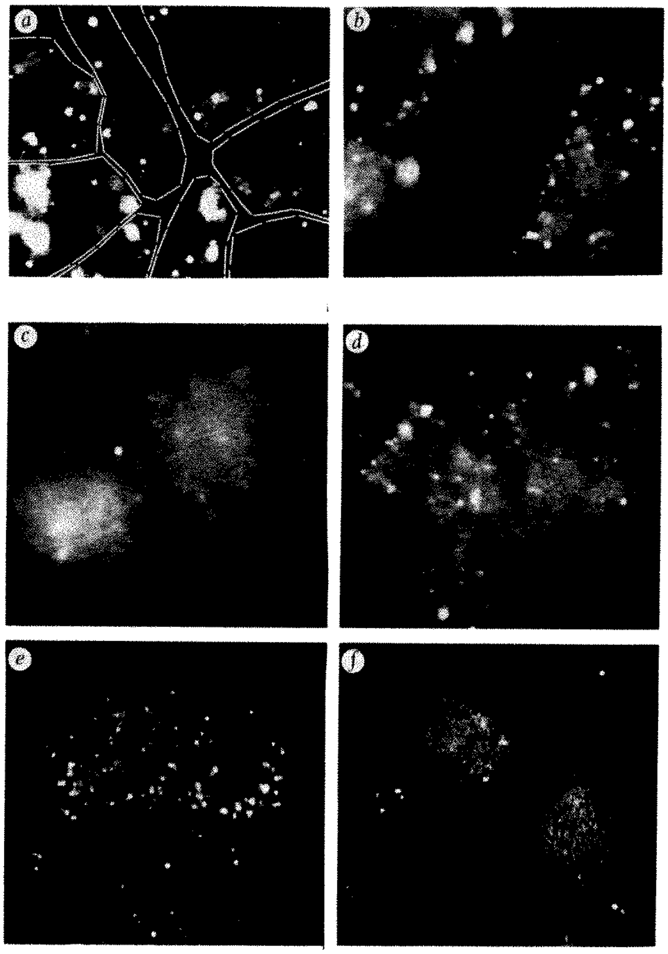

Fig. 2 Effect of dansylcadaverine on R-α_2M and EGF clustering by CHO and NRK cells. *a*, R-α_2M distribution at 37 °C. Fluorescent spots indicate clustered R-α_2M on the cell surface and internalised fluorescent endocytotic vesicles. Dashed line shows outline of individual cell. *b*, R-α_2M + 20 μm dansylcadaverine; *c*, R-α_2M + 100 μM dansylcadaverine; *d*, R-α_2M + 100 μM dansylcadaverine followed by washout of unbound R-α_2M and dansylcadaverine; *e*, NRK cell + rhodamine-lactalbumin-EGF (R-lac-EGF); *f*, NRK + *R*-lac-EGF + 40 μM dansylcadaverine. CHO cells were plated on Falcon tissue culture dishes and grown for 48 h to confluency in DMEM plus 10% heated calf serum. Dishes were rinsed three times with 37 °C DMEM, and preincubated for 30 min at 37 °C in DMEM with or without dansylcadaverine. After preincubation, 20 μl of R-α_2M (6 mg ml^{-1} in PBS) was added and the incubation continued for 30 min at 37 °C. Normal rat kidney cells (NRK) were treated in the same fashion except that R-lac-EGF[13] (100 ng ml^{-1}) was added. After incubation the media were removed and the dishes rinsed three times with 37 °C DMEM and then fixed for 5 min in 2% formaldehyde in PBS at room temperature. Fixative was removed, the dishes rinsed twice with PBS and then fluorescence examined by epifluorescence illumination in a Zeiss RA microscope equipped with a RCA 1030H silicon intensifier television camera. The fluorescence image on the video monitor was directly photographed using a Polaroid camera. In panel *d*, after incubation with R-α_2M, dishes were rinsed four times with DMEM and then incubated for 30 min at 37 °C in DMEM before fixation. All magnifications ×1,467.

compounds assayed in permeabilised NRK cells, shown by solid squares, will be discussed in the following section. For transglutaminase inhibitors with two components in the inhibition curve, the value of K_i for the high-affinity site was used.

The most potent inhibitor of CHO transglutaminase tested was dansylcadaverine, with a K_i for the high-affinity site of 2.5 μM. As discussed above, 20 μM dansylcadaverine produced a 50% inhibition of R-α_2M clustering (Fig. 3, no. 1). Methylamine was much less effective than dansylcadaverine in inhibiting transglutaminase, with a K_i for the high-affinity site of 0.2 mM; high concentrations of methylamine (10 mM) were required to inhibit clustering (Fig. 3, no. 2). Ethylamine was more potent than methylamine both in the transglutaminase assay and as an inhibitor of clustering (ID_{50} 2 mM) (Fig. 3, no. 3). *n*-Propylamine, isopropylamine and *n*-hexylamine, all of which were equivalent to ethylamine as inhibitors of CHO transglutaminase, were also equivalent in terms of their ability to block R-α_2M clustering and internalisation. Finally, both the novel inhibitors of transglutaminase, bacitracin and tolbutamide, inhibited R-α_2M clustering. Bacitracin (Fig. 3, no. 4) was quite a potent inhibitor, with an ID_{50} of approximately 350 μM. Bacitracin was effective as an inhibitor of α_2M clustering in intact CHO, NRK and Swiss 3T3 cells. The oral hypoglycaemic agent, tolbutamide, blocked α_2M clustering. In line with its low affinity for transglutaminase, quite high concentrations of tolbutamide were required to inhibit clustering; the ID_{50} was 20 mM (Fig. 3, no. 5). There was a clear correlation between the activity of compounds as inhibitors of CHO transglutaminase and their ability to inhibit the aggregation and internalisation of α_2M by CHO cells.

Inhibition of clustering in permeabilised NRK cells

The studies of inhibitors of CHO transglutaminase demonstrated a quantitative correlation between inhibition of the enzyme and inhibition of clustering. However, the inhibitors tested were all compounds capable of diffusing into cells. To examine a wider range of transglutaminase inhibitors, we treated cells with 10% dimethyl sulphoxide (DMSO). In these conditions, cells became permeable to low molecular weight compounds (as judged by a loss of Trypan blue exclusion) but retained the capacity to bind and cluster R-α_2M. These studies were carried out with NRK cells because they adhered to the substratum well enough to permit permeabilisation without detachment of cells.

The requirement for permeabilisation is demonstrated by the studies of putrescine inhibition of clustering (Fig. 4*a–c*). Putrescine is a good substrate for NRK transglutaminase (K_m 8.5 mM). However, it does not readily diffuse into cells[23]. Figure 4*a* shows R-α_2M aggregation in permeabilised cells; clusters of fluorescent ligand are seen. Figure 4*b*, *c* shows that, whereas in an intact cell even 100 mM putrescine does not block clustering, in a permeabilised cell, 20 mM putrescine is sufficient to block R-α_2M clustering.

The development of a permeabilised cell model for receptor-mediated endocytosis allowed us to assay peptide inhibitors of transglutaminase. Liver transglutaminase is inhibited by a variety of lysyl peptides[15] of which *N*-acetyl-L-lysine-*O*-methyl ester is one of the more effective (K_i 0.5 mM). This ester also blocked R-α_2M clustering in permeabilised NRK cells, but it was ineffective in intact NRK cells. The correlation of the ID_{50} (4 mM) in NRK cells and the K_i is shown in Fig. 3, no. 7. Another transglutaminase substrate tested was the glutaminyl peptide, carbobenzoxy-glutaminyl-glycine. This inhibitor differed from other inhibitors tested as it competes for the glutaminyl binding site on transglutaminase. The compound is a weak inhibitor of liver transglutaminase (K_i 7.5 mM)[14] and also a weak inhibitor of R-α_2M clustering (ID_{50} 2 mM). This relationship is shown in Fig. 3, no. 9.

Another type of inhibitor tested was an irreversible site-specific inhibitor of transglutaminase, carbobenzoxy-diazonorvaline-paranitrophenyl ester (Z-DONVPA) (J. Folk, personal communication). Intact NRK cells were preincubated for 15 min at 37 °C with Z-DONVPA and then washed extensively

Table 2 Effect of cell density on α_2M clustering and transglutaminase activity in CHO cells

	Fluorescent clusters per cell (mean ± s.d.)	
Cell density	Control	+10 mM methylamine
Low (0.2 × 10^5 cells cm^{-2})	9.9 ± 5.1	<1
High (1.5 × 10^5 cells cm^{-2})	27.5 ± 11.2	6.9 ± 4.2

CHO cells were treated with R-α_2M (100 μg ml^{-1}) in conditions described in Fig. 1 legend. The total number of bright fluorescent spots associated with each was determined by focusing through the entire cell and counting the spots on a video monitor.

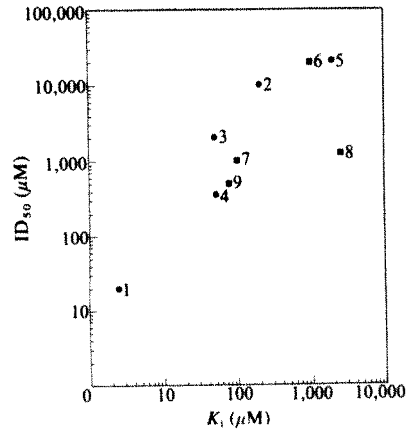

Fig. 3 Correlation between inhibition of transglutaminase and inhibition of α_2-M clustering. Compounds tested were: 1, dansylcadaverine; 2, methylamine; 3, ethylamine, *n*-propylamine, isopropylamine, *n*-hexylamine; 4, bacitracin; 5, tolbutamide; 6, putrescine; 7, *N*-acetyl-lysyl-*O*-methyl ester; 8, *N*-acetyl-(glycyl)$_2$-L-leucyl-L-lysyl-(glycyl)$_2$-glycine; 9, carbobenzoxy-glutaminyl-glycine. ●, Compounds assayed for inhibition of clustering and inhibition of-transglutaminase in CHO cells. The ID$_{50}$ was estimated as the concentration required to produce a 50% decrease in the number and intensity of fluorescent spots and is reproducible within a factor of 2. The K_i was calculated from the formula

$$K_i = \frac{[I]_{0.5}}{1 + (S/K_s)}$$

where $[I]_{0.5}$ was the concentration of inhibitor required to produce a 50% maximal inhibition of enzyme activity. The $[I]_{0.5}$ was derived from the slope of the Eadie–Hofstee plots as shown in Fig. 1*b*, *c*. For dansylcadaverine and methylamine the $[I]_{0.5}$ of the high-affinity component was used. S was the concentration of putrescine in the assay and K_s the Michaelis constant for putrescine; ■, compounds assayed for inhibition of clustering in permeabilised NRK cells (see Fig. 4 legend), K_i value reported for liver transglutaminase[14,15]. Linear regression analysis of the data gave a slope of 0.86, a *y* intercept of 1.3 and a correlation coefficient 0.83. The probability that this correlation was due to chance alone was <0.05.

before being exposed to R-α_2M. Z-DONVPA, 36 μM, in the preincubation step produced complete and irreversible inhibition of R-α_2M clustering.

Stereospecificity of inhibition of α_2M clustering

Folk and his colleagues have synthesised a heptapeptide model substrate of guinea pig liver transglutaminase, acetyl-(glycyl)$_2$-L-leucyl-L-lysyl-(glycyl)$_2$-glycine and have reported a K_i for this compound of 0.08 mM (ref. 15). As shown in Fig. 4*d* low concentrations of this peptide effectively inhibit R-α_2M clustering in permeabilised NRK cells. The ID$_{50}$ for the heptapeptide was estimated to be 0.5 mM (Fig. 3, no. 8). Stereoisomers of the heptapeptide containing either D-lysine (L, D-heptapeptide) or D-leucine and D-lysine (D,D-heptapeptide) are much less active than the L-leucine,L-lysine (L,L-heptapeptide) derivative[15]. As shown in Fig. 4*e*, 1.5 mM D,D-heptapeptide produced no inhibition of R-α_2M clustering. At 8 mM, D,D-heptapeptide was roughly equivalent to 1.5 mM of the L,L derivative (Fig. 4*f*). The L,D-heptapeptide was comparable to the D,D stereoisomer (data not shown). Thus, the sterospecificity of the inhibition of α_2M clustering matches the stereospecificity of guinea pig liver transglutaminase inhibition.

Effects of cell density on R-α_2M clustering

During these studies, it was noted that CHO cells at low cell densities did not produce many fluorescent clusters when exposed to R-α_2M, whereas dense cultures produced many clusters. Birckbichler has reported that the transglutaminase activity of WI-38 fibroblasts rises at confluency[24], and we have noted a precipitous rise in enzyme activity as CHO cells approach confluency. Table 2 compares the number of fluorescent clusters per cell in low cell density and high cell density CHO cells. Confluent cells cluster and internalise α_2M much more efficiently than low density cells. These cells also have much higher transglutaminase levels than low density cells[25]. We do not know if the increase in clustering is directly related to the increased transglutaminase activity, but it is consistent with that interpretation.

Discussion

These studies were undertaken to test the hypothesis that a transglutaminase is involved in receptor-mediated endocytosis of polypeptide hormones and α_2-macroglobulin. The approach was to see if a correlation could be made between the inhibition of transglutaminase and inhibition of the clustering and internalisation of α_2M. Figure 3 clearly demonstrates a remarkable concordance between inhibitors of transglutaminase and inhibitors of α-$_2$M clustering. The inhibitors range in potency over three orders of magnitude and include compounds as diverse as alkyl amines and diamines, lysyl peptides, a glutaminyl peptide, a peptide antibiotic and a sulphonylurea. Linear regression analysis of the data in Fig. 3 gives a slope to the regression line of 0.86, a correlation coefficient of 0.83 and a probability that the two variables are independently distributed of $P < 0.05$. The specificity of the correlation is clearly seen in the comparison of the stereospecificity of the two processes. A heptapeptide containing L-leucine and L-lysine inhibits both transglutaminase activity and R-α_2M clustering much more effectively than do the equivalent L,D or D,D stereoisomers. These observations indicate that it is very likely that a transglutaminase is required for the receptor-mediated endocytosis of certain ligands.

Several points have arisen from these studies. First, not all the transglutaminase activity in cell extracts is essential to hormone internalisation. Only the fraction of activity inhibited by low concentrations of dansylcadaverine, and by low concentrations of methylamine and other alkylamines seems to be related to receptor-mediated endocytosis. A major fraction of the transglutaminase activity in CHO cells was unaffected by most alkylamines, bacitracin or tolbutamide and could only be inhibited by high concentrations of dansylcadaverine or methylamine. These properties were unrelated to inhibition of α_2M clustering. On the basis of its susceptibility to inhibition, we believe the 'essential' transglutaminase activity resembles liver transglutaminase. The other form of the enzyme may be a modified form of liver transglutaminase, a proenzyme or a completely different enzyme. Fractionation of the activities will be required to resolve this question.

A second point is that 5–10 times more inhibitor was required consistently to produce a 50% inhibition of α_2M clustering than was required to produce a 50% inhibition of 'high-affinity' CHO transglutaminase. Several explanations may account for this discrepancy. It could be that 90% inhibition of ligand clustering and internalisation is only perceived as a 50% decrease in the number and intensity of fluorescent spots. Alternatively, 90% inhibition of enzyme activity may be required to interfere with ligand clustering at all. Another possible explanation is that the competition against endogenous substrates in the *in vivo* endocytosis assay may require higher concentrations of inhibitor than the competition against exogenous substrates in the *in vitro* transglutaminase assay. A fourth possibility, which we consider unlikely in view of the striking correlation in Fig. 3, is that receptor-mediated endocytosis requires an enzyme that is not transglutaminase but shares a very similar affinity for the wide variety of inhibitors tested. Although the discrepancies in ID$_{50}$ and K_i values cannot be ignored, we do not think they refute the demonstrated relationship between transglutaminase and endocytosis.

Another point which has developed from these studies concerns the location of the transglutaminase activity essential for α_2M aggregation. Inhibitors such as aliphatic amines or large aromatic molecules (such as dansylcadaverine) would be expected to diffuse readily into cells and these compounds were active in intact cells. Diamines and peptides, on the other hand, were active only after the cell had been permeabilised. This

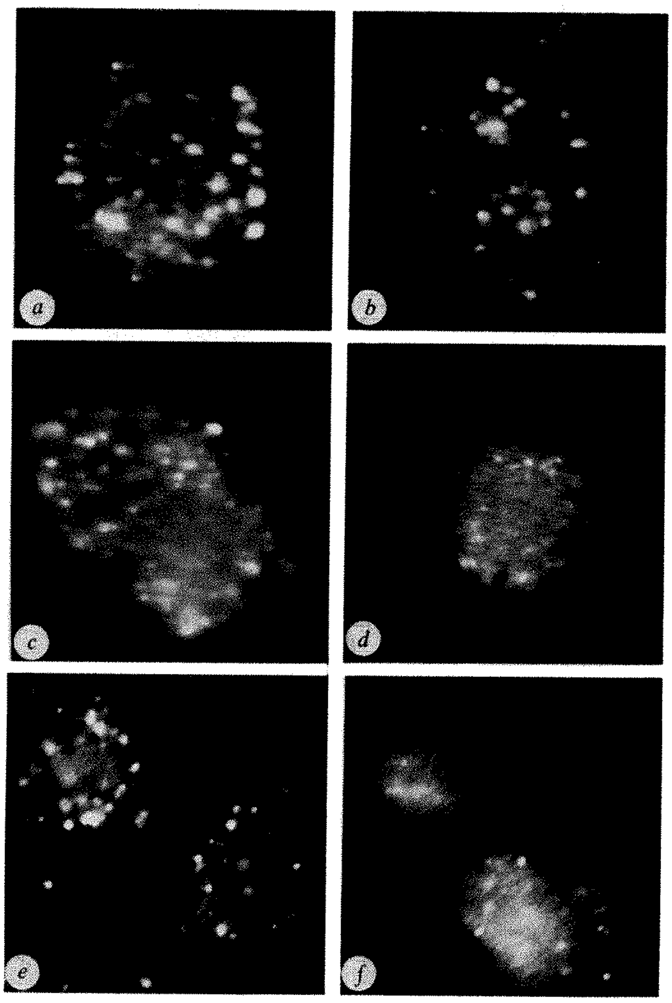

Fig. 4 Inhibition of R-α_2M clustering in permeabilised NRK cells, and effect of putrescine and stereoisomers of a lysyl peptide. *a*, R-α_2M clustering on permeabilised NRK cells; *b*, R-α_2M clustering in the presence of 100 mM putrescine on non-permeabilised NRK cells; *c*, inhibition of R-α_2M clustering by 20 mM putrescine on permeabilised NRK cells; *d*, inhibition of R-α_2M clustering by 1.5 mM acetyl-(glycyl)$_2$-L-leucyl-L-lysyl(glycyl)$_2$-glycine (L,L-isomer) on permeabilised NRK cells; *e*, R-α_2M clustering in the presence of 1.5 mM Ac-(glycyl)$_2$-D-leucyl-D-lysyl-(glycyl)$_2$-glycine (D,D-isomer) on permeabilised NRK cells, *f*, R-α_2M clustering in the presence of 8 mM Ac-(glycyl)$_2$-D-leucyl-D-lysyl-(glycyl)$_2$-glycine (D,D-isomer) on permeabilised NRK cells. Dishes containing normal rat kidney fibroblasts (NRK cells) were rinsed twice with DMEM at 37 °C containing 10% DMSO and then incubated at 37 °C for 30 min in the same media supplemented with inhibitor. The dishes were then gently rinsed twice with warm (37 °C) DMEM and incubated at 37 °C for 20 min in DMEM containing R-α_2M (40 μg ml^{-1}) and the same concentration of inhibitor as in the preincubation. Dishes were then washed, fixed and observed under epifluorescence illumination as described in Fig. 2 legend. All magnifications ×972.

observation suggests that the active transglutaminase is inside the plasma membrane. Furthermore, localisation with anti-guinea pig liver transglutaminase antibodies in 3T3 cells have shown that a cross-reactive antigen (presumably transglutaminase) is located close to the inner aspect of the plasma membrane in the submembrane microfilament mat[26]. Therefore, it seems likely that aggregation of cell-surface receptors somehow involves activity of an enzyme located on the cytoplasmic side of the membrane.

Figure 5 presents a model for receptor-mediated endocytosis which includes a possible role for transglutaminase. Unoccupied receptors for hormones and α_2M are diffusely distributed on the cell membrane. We suggest that in these conditions, transglutaminase in the cell is largely inactive because steady-state intracellular Ca^{2+} levels are far below the levels required for enzyme activity (0.1–1 mM)[27]. Following binding of ligand, occupied receptors may be altered so that they become associated in coated pits. We speculate that formation of small reversible aggregates in coated pits may promote local Ca^{2+} influx and local activation of transglutaminase. Recently, Novogrodsky *et al.*[28] have reported that the binding of concanavalin A to lymphocytes rapidly activates transglutaminase. The presence of activated transglutaminase, elevated Ca^{2+}, and aggregated ligand–receptor complexes might then result in covalent cross-linking of proteins in the complex. The proteins that might be cross-linked by such a reaction are not known. Receptors might be coupled to intrinsic membrane proteins or cytoskeletal proteins or coupled to each other. Recently, Baker *et al.*[29] and Linsley *et al.*[30] have reported covalent cross-linking of EGF, insulin and thrombin to their receptors during receptor-mediated endocytosis. The net effect of covalently cross-linking receptor proteins would be to make the aggregation process irreversible, and would result in a progressive accumulation of ligand–receptor complexes in the coated pit. Ultimately, the pit would pinch off and be internalised, resulting in the endocytosis of ligand–receptor complexes. The crucial step to confirming this hypothesis would be the identification of specific cross-linked proteins generated during receptor-mediated endocytosis.

This hypothetical scheme may not be applicable to all forms of receptor-mediated endocytosis or all routes of hormone internalisation. Some receptors, such as the LDL receptor, aggregate over coated pits even in the absence of ligand binding[1]. Protein cross-linking may not be required to stabilise these ligand–receptor complexes. In a human carcinoma cell line, A431, on the other hand, most EGF–receptor aggregates form over non-coated membrane and may be internalised by pinocytosis or by other mechanisms different from receptor-mediated endocytosis[11]. This form of hormone internalisation may not require transglutaminase activity. The biological significance of these different routes of endocytosis is not clear, but it is possible that cross-linking of proteins during internalisation may be of particular importance for receptor-mediated endocytosis that is coupled to receptor down-regulation.

In these studies, we have used pharmacological manipulation to alter some of the activities of transglutaminase in cells and have observed a concordant change in the ability to aggregate and internalise receptor-bound ligands. However, transglutaminase activity is under physiological control. As the data

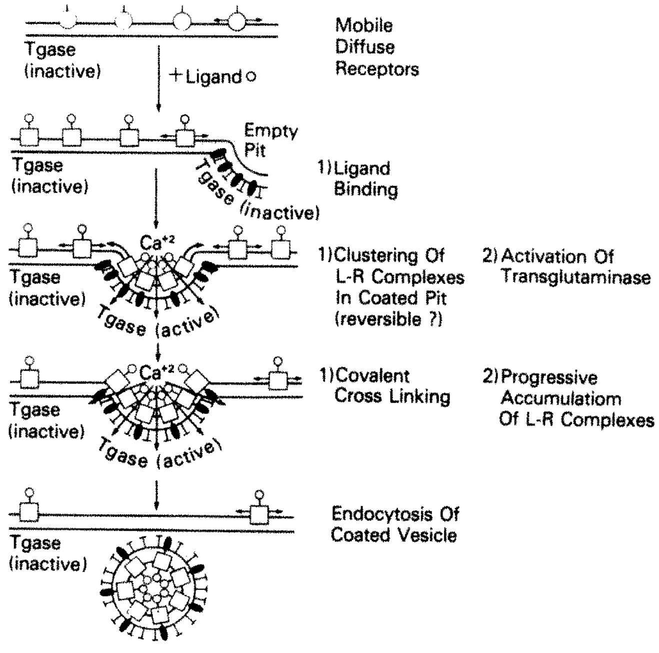

Fig. 5 Hypothetical model for receptor-mediated endocytosis of α_2M and polypeptide hormones. The steps in the model are described in the text. Tgase, transglutaminase.

in Table 2 demonstrate, changes in cell density are correlated with changes in ligand clustering and endocytosis. Transglutaminase activity in cells varies with cell density[24], with changes in intracellular cyclic AMP[25] and in response to viral transformation[24]. Such alterations in transglutaminase activity may be reflected in an alteration in the ability of cells to internalise surface-bound ligands. Maxfield *et al.*[31] have shown that blocking EGF internalisation with inhibitors of transglutaminase produced marked stimulation in the mitogenic response of cells to the hormone. Changes in transglutaminase activity may therefore profoundly modify the way that cells respond to hormones.

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Mapping of metabolites in whole animals by ^{31}P NMR using surface coils

Joseph J. H. Ackerman, Thomas H. Grove, Gordon G. Wong, David G. Gadian & George K. Radda

Department of Biochemistry, Oxford University, South Parks Road, Oxford, UK

The metabolic state of skeletal muscle and brain within intact rats is monitored using high resolution phosphorus nuclear magnetic resonance. Regional disturbances in metabolism (for example, localised ischaemia) are easily observed, indicating the diagnostic possibilities of the method. Measurements are made using 'surface' radiofrequency coils, which we discuss in detail.

IT was demonstrated in 1974 that phosphorus nuclear magnetic resonance (^{31}P NMR) can be used to observe the major phosphorus-containing metabolites in intact, excised muscle[1]. Since then the method, which is non-destructive, has been used to study a variety of biochemical, physiological and medical problems in skeletal muscle[2,3], heart[4,5], kidney[6], liver[7] and brain[8] (for reviews see refs 9–11). Apart from the measurements on mouse brain[8] and the recent experiments on heart in open-chested rats[12,13], all these studies were done on excised organs, albeit often in a physiologically functional state.

Recently, 'spin-imaging' of water in living systems has demonstrated the ability of NMR to obtain spatial discrimination[14]. The combination of high resolution ^{31}P NMR with some method of spatial selection could offer a unique opportunity for the study of metabolism in defined tissues and organs of an intact animal. However, because of the low sensitivity and difficulties with spectral resolution, many of the methods used in water imaging are not yet practical for ^{31}P spin-imaging of living systems. We describe here a simple method for detecting compounds in localised regions adjacent to the surface of the sample. We have used an unusual type of radiofrequency coil, which we call 'surface coil', to obtain the NMR signal. In this article we discuss the theory of the method, describe experiments on test phantoms, and show the application of the technique to the study of muscle and brain metabolism in living animals.

Theory and design of surface coils

The design of radiofrequency (r.f.) coils is best considered in terms of the theory of Hoult and Richards[15]. Let us consider a single r.f. coil that serves the role of both transmitter and receiver. Their theory shows that:

(1) The signal-to-noise ratio resulting from a 90° r.f. pulse applied to a small sample at point q is proportional to the value of $(B_1)_{xy}$ at that point. $(B_1)_{xy}$ is the component of the r.f. magnetic field in the xy plane (that is, in the plane perpendicular to the static field B_0) that is generated by unit current passing through the coil.

(2) The 90° pulse length for a sample at any point q is inversely proportional to the value of $(B_1)_{xy}$ at that point.

(3) The signal-to-noise ratio resulting from a single r.f. pulse of angle ϕ is proportional to $(B_1)_{xy} \sin \phi$. ϕ is equal to $\gamma (B_1)_{xy} i \tau$ where τ is the length of the pulse, γ is the magnetogyric ratio of the nucleus, and i is the current passing through the coil.

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Clearly the field distribution $(B_1)_{xy}$ produced by the coil is a critical factor in its performance. Conventional saddle-shaped and solenoidal coils are generally designed to produce a homogeneous B_1 field throughout the sample volume; this ensures that all regions of a uniform sample contribute equally to the signal. In contrast, we show that a different coil design enables us to observe signals from a selected region adjacent to the surface of the sample.

Consider a single-turn flat circular coil orientated as in Fig. 1. The magnetic field generated at any point q by unit current flowing in the coil is given by Smythe[16] and is

$$B_\rho = \frac{\mu}{2\pi}\frac{x}{\rho[(a+\rho)^2+x^2]^{1/2}}\left[-K+\frac{a^2+\rho^2+x^2}{(a-\rho)^2+x^2}E\right] \qquad (1)$$

$$B_x = \frac{\mu}{2\pi}\frac{1}{[(a+\rho)^2+x^2]^{1/2}}\left[K+\frac{a^2-\rho^2-x^2}{(a-\rho)^2+x^2}E\right] \qquad (2)$$

B_x and B_ρ are the axial (x) and radial (ρ) components of the field at point q, a is the coil radius, μ the permeability of the medium about the coil and K and E are complete elliptic integrals of the first and second kind.

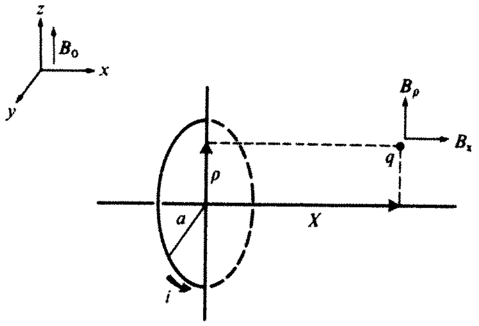

Fig. 1 Diagram illustrating the orientation of a single loop coil. Various parameters are B_0, the main magnetic field, a, the coil radius, i, the current flowing in the coil, and q, a point in space described by the coordinates (ρ, x). B_ρ and B_x are the B_1 field components at q resulting from current i. The angle θ formed by the vector ρ and B_0 is shown as 0 for clarity of presentation.

From Fig. 1, we see that the direction of B_x is always perpendicular to that of the static field B_0, while the perpendicular component of $B_\rho[(B_\rho)_{xy}]$ is given by

$$(B_\rho)_{xy} = B_\rho \sin\theta \qquad (3)$$

where θ is the angle between the directions of B_ρ and B_0 (see Fig. 1 where $\theta = 0$ for clarity of presentation). The magnetic field $(B_1)_{xy}$ generated at any point q therefore depends on θ and is given by

$$(\overline{B_1})_{xy} = \overline{B_x} + (\overline{B_\rho})_{xy} \qquad (4)$$

A plot of $(B_1)_{xy}$ as a function of ρ derived from equations (1)–(4) is given in Fig. 2 for the two extremes $\theta = 0$ and $\theta = \pi/2$.

Following from the relationships given above (points (1) to (3)), it is possible to map the $(B_1)_{xy}$ field of any r.f. coil. We have mapped $(B_1)_{xy}$ for a single-turn flat circular coil of radius 0.75 cm by placing a small sample (a disk of volume 0.01 ml containing 12 M phosphoric acid and 4 mM $NiCl_2$) at different positions q relative to the coil. The field $(B_1)_{xy}$ as determined from the variation in signal intensity is plotted in Fig. 2 and, as can be seen, shows good agreement with theory. Similar agreement was obtained from the variation in 90° pulse length, and from experiments with other surface coils. Note that the ^{31}P test sample was surrounded by an aqueous medium of 154 mM NaCl to approximate the electrical characteristics of animal tissue.

$(B_1)_{xy}$ is localised in a volume about the centre of the coil roughly defined by the coil radius (Fig. 2). It should therefore be possible to observe high resolution NMR signals from selected regions adjacent to the surface of the samples. Furthermore, we should stress that the region of sample contributing significant signal intensity will be spatially far more sharply defined than $(B_1)_{xy}$ due to the sine term in point (3) above.

Finally, as Hoult and Richards[15] have suggested, variations in phase of $(B_1)_{xy}$ should not affect this type of single coil experiment. This is because phase changes induced at transmission are mirrored at reception, and thus cancel. This conclusion was confirmed by our experiments.

Experiments with phantoms

To verify and more clearly illustrate the spatial resolution of surface coils, ^{31}P NMR experiments were performed on two different phantom samples. The first sample consisted of a two compartment rectangular box (10×2×1 cm) partitioned by a thin panel halfway along the length (5 cm) and filled with a solution of Na_2HPO_4 (100 mM) in one compartment and phosphocreatine (100 mM) in the other. ^{31}P NMR spectra were collected using a flat four-turn surface coil (mean radius 0.55 cm) placed outside the box at various positions along its length. The results are shown in Fig. 3. With the coil placed directly over the dividing partition, position 0.0, both compartments contribute equal signal intensity. When the centre of the coil is one radius from either side of the partition, only that compartment directly beneath the coil gives a signal. Thus sample selectivity (as a function of the radial, ρ, coordinate) is approximately confined to an area bounded by the coil circumference.

The second phantom consisted of a box (4×3×3 cm) filled with an aqueous solution of NaH_2PO_4 (20 mM) and NaCl (100 mM). The same coil was placed on the outside of the box (wall thickness 0.1 cm) and the ^{31}P spectrum obtained. A hollow

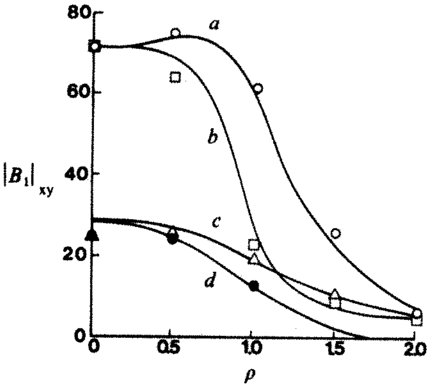

Fig. 2 The spatial dependence of $(B_1)_{xy}$ as a function of ρ, the radial coordinate. ρ is expressed in units of radius, a, while $(B_1)_{xy}$ is normalised to 100 at the centre of the coil, $(\rho, x) = (0, 0)$. The solid curves were derived from equations (1)–(4) while the symbols represent experimental data. Spatial parameters vary for each curve as follows: curve a, $\theta = \pi/2$ and $x \simeq 0.5a$; curve b, $\theta = 0$ and $x \simeq 0.5a$; curve c, $\theta = \pi/2$ and $x \simeq 1.1a$; curve d, $\theta = 0$ and $x \simeq 1.1a$.

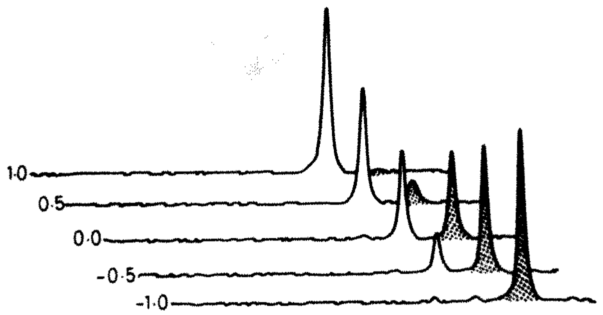

Fig. 3 Stacked plot demonstrating the radial, ρ, resolution of surface coils. The two ^{31}P resonances, each arising from a separate compartment in the phantom, are phosphocreatine (shaded) and inorganic phosphate (Na_2HPO_4) (non-shaded). The label associated with each spectrum is the distance (in units of coil radius, a) between the surface coil centre and the partition which separates the two compartments.

glass disk of radius 0.75 cm and thickness 0.5 cm filled with a solution of NaCl (154 mM) was then placed in the solution-filled box, immediately in front of the coil. On repeating the measurement, a sixfold reduction in signal intensity was observed. This results from the exclusion of 0.8 ml of the NaH_2PO_4 solution in front of the coil, illustrating the three-dimensional spatial selectivity of this type of NMR coil.

These experiments verify the theoretical predictions (Fig. 2) and the properties of these coils can be summarised as follows: (1) The high resolution NMR experiment can be performed with a surface coil external to the sample; (2) the spatial resolution is well defined and can be theoretically and experimentally determined; (3) this spatial resolution may be adjusted by appropriate choice of coil radius and pulse width; (4) the filling factors of surface coils compare favourably with those of conventional coils; and (5) noise generated by r.f. losses in conducting samples[17] arise only from those regions of the samples that contribute signal. These latter two points suggest that good signal-to-noise ratios can be expected with well-designed surface coils.

Measurements of metabolites in whole animals

The results and calculations presented above, together with studies on tissues and perfused organs provided the rationale for attempting to observe ^{31}P NMR spectra from muscle and brain of an intact rat.

Figure 4*a* shows the ^{31}P NMR spectrum obtained by placing the four-turn surface coil against the leg of an anaesthetised rat. The resonances arise from the three phosphates of ATP, phosphocreatine and inorganic phosphate (P_i), within the leg muscle. ^{31}P signals contributed by blood within the muscle tissue are negligible as fresh blood contains only a small amount of ATP and no phosphocreatine relative to 2,3-diphosphoglycerate[18]. (If 2,3-diphosphoglycerate were present in significant quantities, it would produce two signals with chemical shifts of about 5 and 6.5 p.p.m.)

To achieve conditions where peak areas are directly proportional to the amounts of tissue metabolites, a 12-s delay was used between sampling pulses. (This follows from the ^{31}P T_1 relaxation times for intracellular β-ATP (~1 s), phosphocreatine (~3 s) and inorganic phosphate (~3 s).) Thus the spectral accumulation of 128 scans required about 25 min. However, by reducing the delay between pulses, satisfactory spectra can be recorded in less than a minute. These can be used to follow changes in metabolites, to compare different metabolic states, or even to perform quantitative studies if the appropriate relaxation times or saturation factors are accurately determined[10].

A measure of the ATP concentration is obtained from the area of the signal at −16 p.p.m. (the β-phosphate peak of ATP). The resonance at −2.4 p.p.m. contains contributions from the γ-phosphate of ATP and the β-phosphate of mobile ADP. As the ratio of the areas of these two peaks ([ATP]/[ADP]+[ATP]) is one, within experimental error (0.94, $n = 5$, s.e.m. ± 0.05) clearly the concentration of mobile ADP is too low to be observed. The phosphocreatine to ATP ratio is 4.04 ± 0.18 ($n = 5$) which compares reasonably with recent freeze clamping values for rat skeletal muscle[19] but is considerably higher than other reported values[20]. Because phosphocreatine is rapidly degraded during ischaemia[1] we suggest that our *in vivo* ratio of phosphocreatine to ATP represents the best estimate so far for this parameter that reflects the energy status of the cell. The measurement of inorganic phosphate by freeze extraction procedures provides special difficulties, and it is significant that the NMR results give a phosphocreatine: P_i ratio of 14.6 ($n = 5$; s.e.m. ± 1.20). Assuming a phosphocreatine concentration of 23.1 μmol per g wet wt (ref. 19), the P_i concentration derived from NMR is 1.6 μmol per g wet wt, a value considerably lower than that derived from freeze clamping techniques[20]. This observation might explain why the activity of phosphorylase *b* in resting muscle is considerably less than that predicted by studies on the isolated enzyme combined with metabolic measurements by freeze extraction[21].

Following the observations of Moon and Richards[22] it has been well established that the position of the inorganic phosphate resonance provides a good measure of intracellular *p*H

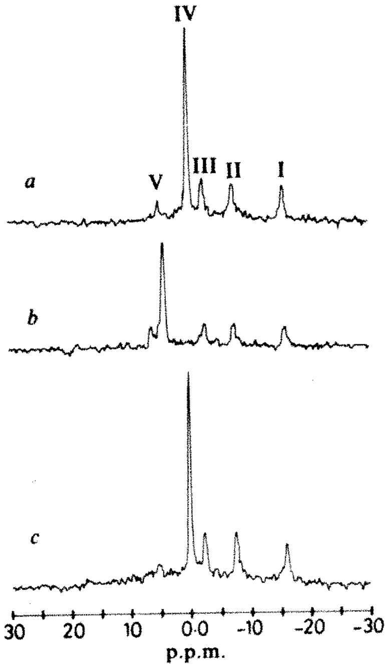

Fig. 4 ^{31}P NMR spectra of the leg muscle of an intact rat obtained with a surface coil. Each spectrum represents 128 scans over 25 min. A 15-Hz line-broadening exponential multiplication was used to enhance signal-to-noise. *a*, Non-ischaemic muscle below the knee joint; *b*, ischaemic muscle from the same area as in *a* after application of a tourniquet just above the knee; *c*, spectrum of muscle above the tourniquet. Chemical shift assignments for non-ischaemic rat leg muscle are β-phosphate of ATP (I), −16.10 ($n = 5$, s.e.m. ± 0.02), α-phosphate (II), −7.54 ($n = 5$, ±0.00), γ-phosphate of ATP (III), −2.47 ($n = 5$, ±0.02), phosphocreatine (IV), 0.00 by definition, and inorganic phosphate (V), 4.90 ($n = 5$, ±0.03). Chemical shifts are defined as positive in the high frequency direction and phosphocreatine is taken as an internal reference. Normally fed, pentabarbital anaesthetised male Wistar rats were used for all experiments. The rats were examined while in a vertical position. All experiments were done at 73.8 MHz on a spectrometer equipped with a wide-bore magnet.

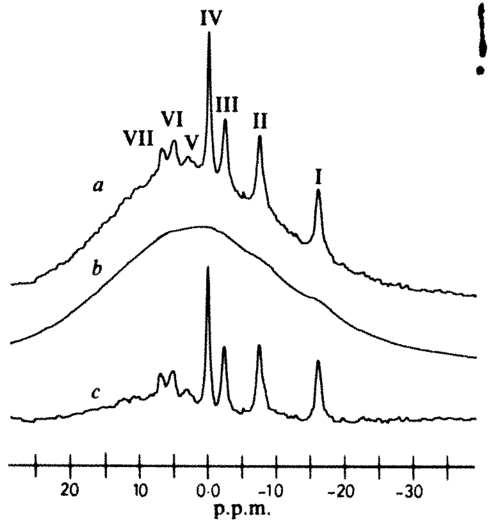

Fig. 5 ^{31}P NMR spectra of the brain of an intact rat obtained with a surface coil. Spectra *a–c* demonstrate the effects of the convolution difference technique in eliminating the broad ^{31}P component arising from bone. *a*, Original brain spectrum, 300 data accumulations over 60 min with a 15-Hz line broadening exponential multiplication; *b*, same as *a* except a 400-Hz line broadening exponential multiplication was used; *c*, difference spectrum of *a* minus *b*. Chemical shift assignments are β-phosphate of ATP (I), -16.20 ($n = 5$, ± 0.05), α-phosphate of ATP (II), -7.62 ($n = 5$, ± 0.02), γ-phosphate of ATP (III), -2.48 ($n = 5$, ± 0.02), phosphocreatine (IV) 0.00 by definition, phosphodiesters (V) 3.0 ($n = 3$, ± 0.1), inorganic phosphate and the 2-phosphate of 2,3-diphosphoglycerate (VI), 5.00 ($n = 5$, ± 0.007), and sugar phosphates and the 3-phosphate of 2,3-diphosphoglycerate (VII), 6.65 ($n = 5$, ± 0.06).

(refs 9, 10). The chemical shift of P_i within rat leg muscle is 4.90 ± 0.03 ($n = 5$), consistent with a *p*H of 7.1 ± 0.02 ($n = 5$).

In an experiment similar in philosophy to the two compartment phantom, the effect of localised ischaemia in the rat leg muscle was assessed (Fig. 4). A control muscle spectrum was obtained from just below the knee joint (Fig. 4*a*). Then a very tight tourniquet was placed around the leg just above the knee and a spectrum obtained from the same location as the initial control (Fig. 4*b*). The two spectra are significantly different. The ischaemic region of the muscle has no phosphocreatine, somewhat reduced amounts of ATP and a very large P_i concentration. In addition, the P_i chemical shift (4.31 p.p.m.) differs significantly from that in the control muscle (4.92 p.p.m.), indicating a more acidic *p*H of 6.7. (Similar results with excised tissue have been observed[1–3].) The small new peak at 6.30 p.p.m. may be attributable to the build-up of sugar phosphates or nucleotide monophosphate. Finally, with the tourniquet still in place, a spectrum was obtained from the leg muscle above the tourniquet (Fig. 4*c*). This spectrum is very similar to that of the first control, indicating healthy, non-ischaemic muscle. The localisation of ischaemic areas in diseased muscle is thus clearly possible.

One of the most difficult organs to study by biochemical and perfusion techniques is the brain. Because of the rapid oxidative metabolism and sensitivity to short periods of hypoxia or ischaemia of the brain, considerable effort has been spent in developing a variety of techniques such as 'freeze-blowing'[23], and cryo-NMR[8] to assess the *in vivo* energy status of this tissue.

By placing a surface coil on top of the rat's head, directly over the brain, we were able to obtain excellent ^{31}P spectra from the brain (Fig. 5). As there is no muscle tissue between the coil and the brain these experiments are selective for brain tissue and bone only. The very broad contribution from the bone can easily be eliminated by the convolution difference technique[24].

From the chemical shifts of the three ATP resonances in brain and muscle we can conclude that in both tissues the ATP is predominantly complexed to divalent cations which we assume to be Mg^{2+}.

The energy status of the live brain may be best compared with that of perfused or rapidly frozen tissue from the value of the phosphocreatine to ATP ratio. The value we derived from NMR (1.93 ± 0.12, $n = 5$) is higher than the best values obtained by rapid freezing[23]. In addition we estimate that the concentrations of both mobile ADP and P_i are much lower than those measured by destructive analytical techniques. In the brain spectrum the ratio of the resonances β-phosphate of ATP: γ-phosphate of ATP + β-phosphate of ADP is 1.07 ± 0.08 ($n = 5$) indicating that ADP is not detectable. The P_i signal is obscured by the 2-phosphate peak of 2,3-diphosphoglycerate present in blood. The 3-phosphate from the same compound is at 6.6 p.p.m. and indicates that the P_i concentration in the brain must be well below that of ATP, in contrast to values obtained from freeze extraction. These considerations lead to the conclusion that the phosphorylation state in brain tissue ($[ATP]/[ADP][HPO_4^{2-}]$) is at least an order of magnitude higher than the generally accepted value of $\sim 3{,}400\ M^{-1}$.

Conclusions

The use of surface coils to obtain high resolution NMR spectra from selected regions of large objects is clearly not limited to the systems described here. The design of the coil can be adapted to suit the sample. In animals, tissues other than muscle and brain (for example, fat tissue or cancerous growth) could be studied. Coupled with surgery, internal organs can easily be investigated[12].

An early low resolution application of flat coils has been for the measurement of blood flow[25]. Plant, fungal or non-living materials can also be examined in this way.

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Influenza antigenic determinants are expressed from haemagglutinin genes cloned in *Escherichia coli*

J. S. Emtage, W. C. A. Tacon, G. H. Catlin, B. Jenkins, A. G. Porter & N. H. Carey

Searle Research Laboratories, Lane End Road, High Wycombe, Bucks, UK

A gene sequence for the fowl plague virus (FPV) haemagglutinin molecule has been inserted into a bacterial plasmid such that its transcription is under the control of a promoter derived from the tryptophan operon. Such plasmids direct the synthesis of a protein that reacts specifically with antisera to FPV haemagglutinin. Evidence is also presented that in some cases DNA inserted at the Hind*III site of pBR322 is expressed.*

INFLUENZA is still a major disease of man. It causes death primarily in the elderly, many of whom have chronic heart or vascular disease and other disorders. Severe infections of healthy individuals also occurs but in these cases morbidity, leading to incapacity to work, rather than mortality usually results. The morbidity figures however are of great economic importance. For example, the Hong Kong outbreak of 1968–70 resulted in 25 million lost working days in England[1], while in the US the effect of the Hong Kong variant of 1968–69 was costed at 3.8 billion dollars[2]. Thus, prevention of influenza epidemics and pandemics would be of value both economically and socially.

The main structure involved in immunity against influenza is the haemagglutinin (HA) surface glycoprotein[3,4]. The functional HA subunit, one of the spikes on the virus surface, is a triangular rod-shaped glycoprotein with a molecular weight of ~250,000 and is comprised of three HA monomers[5]. The HA monomers are synthesised as single polypeptide chains containing, in the case of FPV, an 18-amino acid precursor peptide at the N-terminus[6]. During maturation and virus assembly the pre-HA is further processed to HA1 and HA2 which remain linked by disulphide bridges[7,8].

Recent advances in genetic engineering and our knowledge of the structure and sequence of bacterial operons and control elements now allow the construction of new bacterial strains with the potential of synthesising large quantities of viral proteins (antigens) which may ultimately be useful as vaccines. In this article we describe the controlled production of haemagglutinin antigen from an HA gene cloned in *Escherichia coli*; this is the first step towards testing the feasibility of large scale antigen production by these means.

Experimental design

We have previously cloned at the *Hind*III site of pBR322 a DNA fragment containing the control region of the *E. coli* tryptophan operon, and coding for the ribosome binding site and first seven amino acids of *trpE*. From this, we have produced a series of vectors, pWT111, pWT121 and pWT131, capable of ensuring that any inserted DNA is read in the correct phase[9].

We recently cloned and sequenced the gene for the FPV haemagglutinin protein[6]. The gene was cloned using *Hind*III linkers and can therefore be transferred between vectors. From the nucleotide sequences of the FPV HA gene and the *Hind*III sites of the pWT series, it was clear that, if inserted at the *Hind*III site of pWT121 in the correct orientation, the HA gene would be translated by readthrough from the *trpE* fragment (Fig. 1*A*).

The protein resulting from initiation at the *trpE* AUG would be a hybrid consisting of the following fragments in order: (1) an N-terminus of 7 amino acids from anthranilate synthetase; (2) 6 amino acids specified by linker DNA; (3) 6 phenylalanine residues from the $(T)_{19}$ region of the FPV cloned DNA; (4) 7 amino acids from the normally non-translated 5′-portion of the HA gene; (5) 558 amino acids comprising the haemagglutinin protein and its prepeptide[6] and, finally; (6) 5 amino acids specified by *Hind*III linker at the C-terminus[9]. This is a total of 589 amino acids with a total MW of 69,000.

It was also clear that the HA gene should not be expressed from the initiator AUG of the *trpE* fragment in pWT111 or pWT131. Recognition of the ribosome binding site and initiator AUG of the HA was not anticipated. Similarly, we did not expect expression of the HA gene inserted at the *Hind*III site of pBR322 in either orientation.

Construction of expression plasmids

pWT121 was restricted with *Hind*III, treated with alkaline phosphatase, ligated to the purified HA gene and the mixture used to transform *E. coli* K12 HB101. A total of 47 ampicillin resistant transformants was obtained (pWT121 in the absence of the HA gene produced 13 colonies). Of these 47 colonies, 19 were tetracycline sensitive (Tc^S) and the remainder tetracycline resistant (Tc^R). We selected 12 colonies ($3Tc^S$ and 9 Tc^R) for further characterisation. Analysis of plasmid DNA by gel electrophoresis after *Hind*III restriction showed that 5 of the 9 Tc^R colonies contained plasmids with a DNA insert. The other four contained the parent plasmid.

The orientation of the inserted DNA was determined by restriction enzyme analysis of the plasmids. We denote the two orientations as either R or L and define R and L orientation in terms of the direction of transcription required for HA

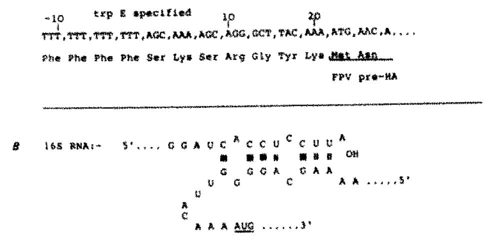
A
pWT111:- ATG,CAA,ACA,CAA,AAA,CCG,ACT,CCA,AGC,TT.......
Met Gln Thr Gln Lys Pro Thr, Pro Ser
trp E specified
pWT121:- ATG,CAA,ACA,CAA,AAA,CCG,ACT,CCA,AGC,TCC,AAG,CTT,.......
Met Gln Thr Gln Lys Pro Thr, Pro Ser Ser Lys Leu
trp E specified
10
20
FPV-HA:- AAGCTTGGTTTTTTTTTTTTTTTTTTTAGCAAAAGCAGGGGTTACAAAATG,AAC,A...
Met Asn
So FPV-HA gene cloned into pWT121 in the R-orientation will produce:-
-50
-40
-30
-20
ATG,CAA,ACA,CAA,AAA,CCG,ACT,CCA,AGC,TCC,AAG,CTT,GGT,TTT,TTT,
Met Gln Thr Gln Lys Pro Thr, Pro Ser Ser Lys Leu Gly Phe Phe
-10
trp E specified
10
20
TTT,TTT,TTT,TTT,AGC,AAA,AGC,AGG,GCT,TAC,AAA,ATG,AAC,A....
Phe Phe Phe Phe Ser Lys Ser Arg Gly Tyr Lys Met Asn
FPV pre-HA
B
16S RNA:- 5'.... G G A U C A C C U C C U U A OH
G G G A C G A A A A .....5'
U U A C A A A AUG ......3'

Fig. 1 *A*, Predicted nucleotide sequences around the *Hind*III sites of pWT111, pWT121 and the FPV-HA gene. Arrows indicate the position of the *Hind*III sites and, for clarity, only the sequence of the coding strand is shown. The protein sequence of the N-terminus of anthranilate synthetase has previously been reported by Lee *et al*[10]. The numbering system is bidirectional beginning with the 5′-nucleotide of the gene 4 complementary RNA. *B*, Potential base pairing between the 3′oligonucleotide of *E. coli* 16S RNA[11] and the pre-AUG region of the haemagglutinin mRNA.

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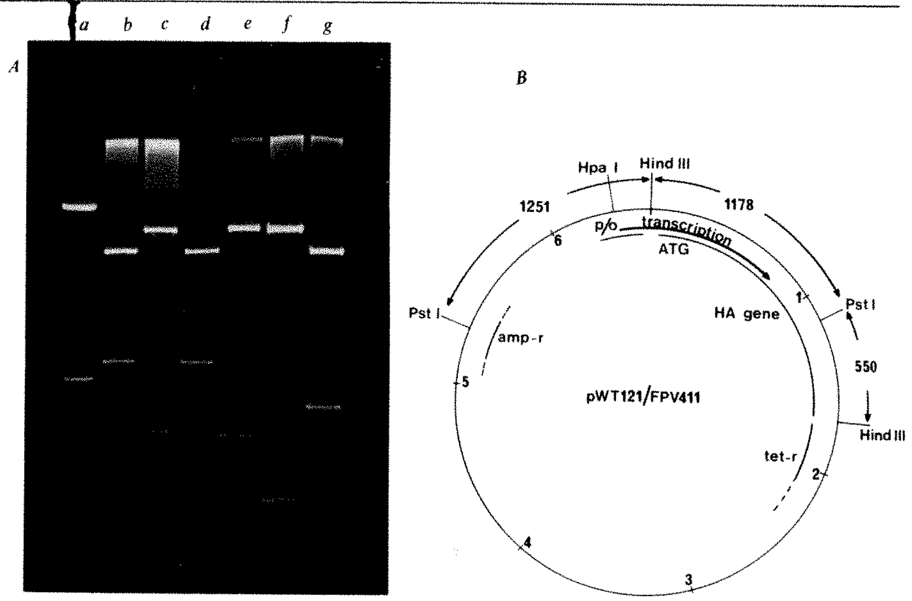

Fig. 2 *A*, Orientation of the FPV gene in pWT111, pWT121 and pBR322. pBR322, pWT111 or pWT121 (10 μg) was limit digested with *Hin*dIII. The resulting 5′-terminal phosphates of the vectors were removed by treatment with 20 μg bacterial alkaline phosphatase for 30 min at 37 °C in a 25 μl incubation containing 20 mM Tris-HCl *p*H 7.5 and 0.1% SDS. After phenol extraction and ethanol precipitation 0.2 μg of each DNA was ligated to ~40 ng FPV-HA gene in a 20 μl incubation containing 50 mM Tris-HCl *p*H 7.5, 10 mM $MgCl_2$, 20 mM dithiothreitol, 1 mM ATP and 0.02 units T4 DNA ligase (New England Biolabs). After overnight incubation at 15 °C the mixtures were diluted to 100 μl with TCM (10 mM Tris-HCl *p*H 7.5, 10 mM $CaCl_2$, 10 mM $MgCl_2$) and used to transfect 200 μl $CaCl_2$-treated *E. coli* K12 HB101 by a previously described procedure[12]. Transformants present after growth for 16 h at 37 °C on L-agar[13], plus 100 μg ml^{-1} carbenicillin (Pyopen) were tested for tetracycline resistance by picking onto agar plates containing M9 salts, glucose and casamino acids[13] plus carbenicillin and tetracycline (10 μg ml^{-1}). For plasmid isolation colonies were picked and 100-ml cultures grown in M9 salts, glucose, casamino acids medium supplemented with carbenicillin. At an A_{600} of ~0.6, choramphenicol was added to a final concentration of 150 μg ml^{-1} and incubation continued for 16 h at 37 °C. Cells were killed by the addition of diethylpyrocarbonate[14] to 0.4%, collected by centrifugation, washed with TE buffer (10 mM Tris-HCl *p*H 7.5, 1 mM EDTA) and suspended in 1.5 ml of iced 25% sucrose in 50 mM Tris-HCl *p*H 8. Cells were lysed by the addition of 0.5 ml lysozyme (10 mg ml^{-1}, 0.5 ml EDTA (0.5 M, *p*H 8) and 2.5 ml iced Triton solution (0.1% Triton X-100, 50 mM Tris-HCl *p*H 8, 50 mM EDTA) at 5-min intervals. The resulting lysates were cleared by spinning at 15,000*g* for 20 min, extracted with phenol and then chloroform and finally precipitated by the addition of 0.1 vol 3M NaAc and 0.54 vol isopropanol. After 30 min at −20 °C the DNA was pelleted, washed with 70% ethanol, dried and dissolved in TE. *Pst*I digests were carried out on 0.1 μg DNA in conditions recommended by the vendor (New England Biolabs) with the modification that RNase (50 μg ml^{-1}) was included in the incubation. Digests were fractionated on a 1.4% agarose slab gel[15] and bands visualised under UV light after staining with ethidium bromide. Lane *a* contains PM-2 DNA digested with *Hin*dIII. Bands seen are 5,400, 2,350 and 1,050 base pairs. The other lanes contain *Pst*I digests of the following plasmids: *b*, pWT121/FPV 411(R); *c*, pWT121/FPV 412(L); *d*, pWT111/FPV 502(R); *e*, pWT111/FPV 503(L); *f*, pBR322/FPV 604(L); *g*, pBR322/FPV 605(R). *B*, Structure of pWT121/FPV 411(R). pWT121 contains a single *Hin*dIII site downstream from the *trp* promoter[9]. The plasmid shown contains the FPV-HA gene cloned at the *Hin*dIII site in the R orientation. The distances between the *Pst*I and *Hin*dIII sites are shown as well as the direction of transcription from the *trp* promoter and the position of the initiator ATG. A full description of the construction of the pWT plasmid series has been published elsewhere[9]. Briefly, the 497-base pair *Hin*fI fragment containing the *trp* promoter/operator region as well as the nucleotides specifying the leader sequence and first seven amino acids to *trpE* (ref. 10) was isolated and cloned, using *Hin*dIII linkers, into the *Hin*dIII site of pBR322. From this a plasmid, pWT101, was isolated containing the *trp* promoter in the R orientation. In pWT101 tetracycline resistance is controlled from the *trp* promoter. Elimination of the *Hin*dIII site upstream of the *trp* promoter produced the vector pWT111 and phase changing at the *Hin*dIII site of pWT111 using DNA polymerase I and *Hin*dIII linkers produced pWT121 and pWT131.

production. That is, a gene in the R orientation required rightward (clockwise) transcription for expression; in the pWT plasmid series this is the orientation for expression from the *trp* promoter. For clarity we have included the letter (R) or (L) after the plasmid number to indicate its orientation. pWT121 contains a single *Pst*I site, in the ampicillin gene[16], 1,251 base pairs from the *Hin*dIII site and the FPV HA gene contains a *Pst*I site 1,178 base pairs from the 5′ end of the coding strand[6]. Thus *Pst*I digests of the recombinant plasmids should indicate which are correctly orientated with respect to the *trp* promoter. Representatives of the two FPV-gene containing groups are shown in Fig. 2*A* (*b* and *c*). The Tc^S plasmids gave rise to a band of 1,800 base pairs on *Pst*I digestion while the Tc^R plasmids produced one of 2,450 base pairs. These are consistent with the fragments expected from the plasmid shown in Fig. 2*B* and indicate that all the Tc^S plasmids contained inserts in the L orientation while the Tc^R plasmids contained inserts in the R orientation.

In a similar way we re-cloned the HA gene into the *Hin*dIII site of pWT111 and pBR322. Again the pWT111/FPV plasmids could be divided into Tc^R and Tc^S groups and *Pst*I restrictions (Fig. 2*A*, *d* and *e*) of representatives of these groups indicated that the HA gene was in the R orientation in the Tc^R group and in the L orientation in the Tc^S group. The pBR322/FPV plasmids however were all Tc^S but still contained L and R orientated genes as shown in Fig. 2*A* (*f* and *g*).

The correct phasing of the HA gene in pWT121/FPV 411(R) with the *trpE* AUG was confirmed by nucleotide sequencing. The HA gene contains an *Eco*RII restriction site at positions 36–40 (ref. 6). This site, however, is methylated and although not cleaved by *Eco*RII, it is cleaved by the *Eco*RII isoschizomer *Bst*N1. Therefore we digested pWT121/FPV 411(R) with *Bst*N1, labelled the 5′ ends with ^{32}P, recut the DNA with *Hpa*I and isolated the fragment containing the initiation region. The nucleotide sequence of this fragment was complementary to the sequence predicted in Fig. 1*A*, except for nucleotide −36 which was an A instead of a G. This was also the case for the corresponding position in pWT111. In both plasmids this position corresponds to the 3′-terminal nucleotide of the *Hin*dIII linker. The reason for the change is not clear; it might be due to random mutation or, more likely, to micro-heterogeneity or incomplete deblocking after chemical synthesis at the 3′ ends of the *Hin*dIII linker. In either event the effect in the mRNA strand is to have a serine codon (UCG) instead of a proline codon (CCG) but does not change the phase with respect to the *trpE* AUG.

Gene expression

E. coli colonies containing representatives of the plasmids described above were screened for FPV-HA antigen using a solid-phase immunological method[17]. Briefly, small cultures of individual colonies were grown, collected and lysed using lysozyme and Triton X-100. Any HA sequences present were

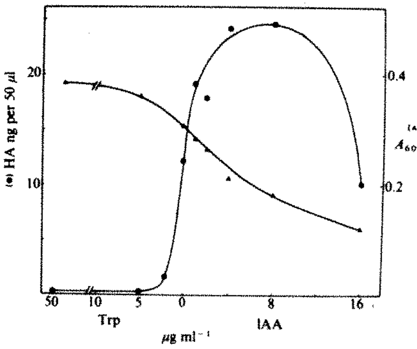

Fig. 3 Control of HA production. Overnight cultures of *E. coli* K12 HB 101 containing pWT121/FPV 411(R) were grown in M9 salts, glucose, casamino acids medium supplemented with carbenicillin (100 μg ml^{-1}). Aliquots of 100 μl were inoculated into 5 ml of the above medium containing either tryptophan or IAA as indicated. Cultures were grown for 2 h at 37 °C, the A_{600} determined and cells collected, lysed and assayed for HA content as described in Table 1.

Table 1 Radioimmunoassay of FPV-HA in lysates of bacteria containing influenza genes

Plasmid	Orientation of gene	Phenotype	HA content (ng per 50 μl)
pWT121/FPV 411	R	Tc^R	20.3
pWT121/FPV 412	L	Tc^S	5.0
pWT111/FPV 502	R	Tc^R	4.1
pWT111/FPV 503	L	Tc^S	2.1
pBR322/FPV 604	L	Tc^S	3.6
pBR322/FPV 605	R	Tc^S	0
pWT121	—	Tc^R	0
pBR322	—	Tc^R	0

Liquid cultures (5 ml) of individual colonies were grown in M9 medium. Cells were collected by centrifugation, and lysed, in a final volume of 1 ml, as described in Fig. 2*A*. This lysate was used for antigen assay without further treatment. HA-antigen assays were performed in polystyrene culture tubes (Nunc no. 1410) using an antibody sandwich technique[17]. Rabbit anti-FPV HA was purified by ammonium sulphate precipitation and DEAE-cellulose chromatography[18] before use and labelled with ^{125}I as described elsewhere[19]. Tubes were coated with 50 μl of 0.2M $NaHCO_3$*p*H 9 containing 60 μg ml^{-1} purified, unlabelled IgG for 10 min at room temperature, washed with 3 × 1-ml aliquots of wash buffer (phosphate-buffered saline containing 0.1% bovine serum albumin, 0.1% NP-40 and 0.5% normal goat serum) and then incubated at room temperature either with known amounts (1–20 ng) of Sarkosyl-disrupted FPV or with the bacterial lysates. After 2 h, the tubes were washed with 4 × 1-ml aliquots of wash buffer and finally incubated at 4°C for 16 h with 50 μl wash buffer containing 200,000 c.p.m. ^{125}I-anti-HA. Tubes were again washed and counted in a Packard γ-counter. Antigen present was quantitated from standard curves relating the radioactivity bound to the amount of FPV present.

bound to a polystyrene tube coated with FPV-HA specific IgG. Bound antigen was then detected by incubating with high specific activity ^{125}I-anti FPV-HA. As indicated in Table 1, immune reactivity was detected from all colonies containing an FPV-HA gene inserted into the pWT plasmids and from pBR322 containing the HA gene in the L orientation. Neither of the parent plasmids nor pBR322 containing the HA gene in the R orientation produced any immune reacting material. Further, the positive reactions were abolished if the tubes were coated either with normal rabbit IgG or with anti A/Victoria—HA IgG instead of specific anti-FPV-HA IgG.

It was interesting to find expression of HA antigen from one orientation of pBR322/FPV. The *Hin*dIII site of pBR322 lies in the promoter region of the tetracycline gene and it is known that cloning at the *Hin*dIII site destroys this rightward transcribing promoter[20] unless the cloned DNA has its own promoter transcribing into the tetracycline gene(s)[21]. We therefore propose that a previously unknown promoter which transcribes in the leftward direction exists on the tetracycline gene side of the *Hin*dIII site of pBR322. It is necessary to postulate the existence of this promoter to explain the expression of the HA gene in pBR322/FPV 604(L), pWT121/FPV 412(L) and pWT111/FPV 503(L). The possibility that there is a pseudopromoter sequence in the HA gene itself, for example the (T_{19}):(A_{19}) sequence, is ruled out as the plasmid pBR322/FPV 605(R) does not express its HA gene (Table 1).

This postulated promoter would explain the transcription of the HA gene in the L-orientated plasmids. How is translation explained? Inspection of the nucleotide sequence around the tetracycline–HA gene junction of pBR322/FPV 604(L) reveals nonsense triplets in all three translation phases[22]. Thus the bacterial translational system is probably recognising a nucleotide sequence on the HA mRNA itself and initiating protein synthesis at the AUG of the pre-peptide. Comparison of the sequence of the untranslated region of the HA gene with that of the 3′ end of prokaryotic 16S ribosomal RNA[11] reveals surprising complementarity (Fig. 1*B*). Consistent with this interpretation is the expression of HA antigen in pWT111/FPV 502(R). In this case the HA gene is in the wrong phase to be translated from the *trpE* AUG; protein synthesis initiating at the *trpE* AUG is terminated by the now in phase UGA triplet at position 23–25 in the HA gene. Thus we conclude that initiation is from the natural HA AUG at position 22–24 (Fig. 1*A*). Assuming equal transcription from the *trp* promoter in pWT121/FPV 411(R) and pWT111/FPV 502(R) and that the products have equal antigenic properties, then from the data of Table 1 we conclude that the eukaryotic ribosome binding site is recognised with an efficiency of 20% compared to the prokaryotic site.

The fivefold difference in HA expression between pWT121/FPV 411(R) and pWT111/FPV 502(R) could be due to differences in the plasmid complement of the respective cells. To exclude this possibility the relative copy numbers of the above plasmids were determined from agarose gels of lysed whole cells[9] and found to be the same (data not shown).

Hitherto the *Hin*dIII and *Eco*RI sites of pBR322 have been assumed to be non-expression sites because of their respective situations in and upstream of the tetracycline promoter[20,21]. This has been thought to be of value in assessing the containment categorisation when considering the safety of experiments in genetic engineering[23]. In view of the above findings it is clear that the validity of these assumptions should be reassessed. It seems likely that a potential ribosome binding site on the inserted gene will be of some importance.

Table 2 Control of HA expresion from the tryptophan promoter/operator region

Plasmid	Addition	HA* (ng per 50 μl)
pWT121/FPV 411(R)	Tryptophan	0.9
	IAA	59.1
pWT121/FPV 412(L)	Tryptophan	9.6
	IAA	4.9

Cultures (5 ml) of pWT121/FPV 411(R) and pWT121/FPV 412(L) were grown for 3 h at 37 °C in M9 salts, glucose, casamino acids medium supplemented with carbenicillin (100 μg ml^{-1}) and either IAA (20 μg ml^{-1}) or tryptophan (100 μg ml^{-1}). Cells were collected, lysed and assayed for HA content as described in Table 1.

* Results normalised to same A_{600}.

Control of gene expression

In vivo the tryptophan operon is controlled by the level of tryptophan[24] and control is best obtained in culture by using tryptophan as repressor or β-indole acrylic acid (IAA) as inducer. The latter is a competitive inhibitor that prevents tryptophan binding and thereby effectively inactivates the repressor[25].

We selected a representative colony from each of the two expressing groups (in pWT121) to examine HA synthesis in the above conditions. Our initial observation was that pWT121/FPV 411(R) grew very slowly in the presence of 20 μg ml^{-1} IAA; there was a threefold difference compared to growth in the presence of tryptophan. pWT121/FPV 412(L) grew equally well in both sets of conditions. The results of radioimmunoassay of these cultures are shown in Table 2. Because of the differences in growth rate, the values are all normalised to the A_{600} of the culture containing pWT121/FPV 411(R) plus tryptophan. In pWT121/FPV 411(R), HA production is stimulated 65-fold when the *trp* operon is derepressed. In contrast, IAA decreased HA synthesis in pWT121/FPV 412(L). Thus increased transcription from the *trp* promoter is competing with transcription from the presumed promoter near or around the tetracycline gene and reducing expression in the leftward direction.

The effects of repression and induction on HA synthesis are also seen in Fig. 3. As little as 5 μg ml^{-1} tryptophan was sufficient for maximal repression. On the other hand, because of its effect on growth, the induction characteristics of IAA were more complex. Maximal induction occurred at IAA concentrations of 4–8 μg ml^{-1} and HA synthesis was stimulated 120-fold over the repressed level.

Finally, we examined the immunoprecipitable products in cells containing various plasmids after induction or repression of *trp* transcription. At 3 h after addition of either IAA or tryptophan to cultures of such cells, aliquots were pulse-labelled with ^{35}S-methionine and the immunoprecipitable proteins separated by SDS-polyacrylamide gel electrophoresis. No immunoprecipitable proteins were present in induced cells containing pWT121 or in repressed cells containing pWT121/FPV 411(R) (Fig. 4*a* and *b*, respectively). However, induced cells containing pWT121/FPV 411(R) or pWT111/FPV 502(R) and repressed cells containing

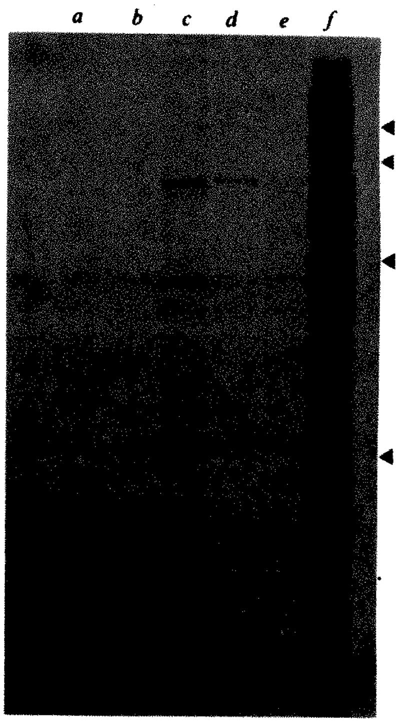

Fig. 4 Immunoprecipitation of the haemagglutinin-like protein. Cultures of *E. coli* K12 HB101 containing the plasmids pWT121, pWT121/FPV 411(R), pWT121/FPV 412(L) and pWT111/FPV 502(R) were grown in M9 salts, glucose, casamino acids medium containing carbenicillin (100 μg ml^{-1}) and either IAA (10 μg ml^{-1}) or tryptophan (40 μg ml^{-1}). After a 3-h period, 5-ml samples were removed and pulse-labelled for 10 min at 37 °C with 50 μCi of ^{35}S-methionine. The labelling was terminated by the addition of 20 ml cold M9 medium, the cells pelleted by centrifugation at 12,000*g* for 10 min at 4 °C and lysed, in a final volume of 1 ml, as described in Fig. 2*A*. Immunoprecipitations were performed as follows: to 400 μl *E. coli.* extract was added 2 μl 10% Nonidet P-40 (NP–40), 4 μl (2μg) normal rabbit IgG and the mixture incubated for 60 min at 20 °C. An aliquot of 10 μl of a 10% suspension of killed *Staphylococcus aureus*[31] (Cowan 1 strain) was then added and incubation continued for a further 15 min. The resulting immunoprecipitate was pelleted and discarded. To the supernatant was added 2 μg rabbit anti FPV-HA and, after 60 min at 20 °C, 10 μl of *S. aureus.* This immunoprecipitate was pelleted 15 min later and washed twice with a solution containing 0.15 M NaCl, 5 mM EDTA, 50 mM Tris-HCl *p*H 7.4 and 0.05% NP-40. Radioactive proteins were eluted from the complex with 30 μl SDS buffer, heated 90 °C for 2 min and electrophoresed on a 12.5% polyacrylamide-SDS gel[32] which was then dried and autoradiographed. The figure shows the immunoprecipitable proteins from cells containing the following plasmids: *a*, pWT121 + IAA; *b*, pWT121/FPV 411(R) + *trp*; *c*, pWT121/FPV 411(R) + IAA; *d*, pWT111/FPV 502(R) + IAA; *e*, pWT121/FPV 412(L) + *trp*; *f* contains an extract from induced cells containing pWT121/FPV 411(R). The positions of conalbumin (MW 76,000), bovine serum albumin (69,000), ovalbumin (43,000) and carbonic anhydrase (30,000) are indicated by the arrows.

pWT121/FPV 412(L) all produced a band of MW 61,000 (Fig. 4*c*, *d* and *e* respectively). Our experimental design predicted that the HA-like protein synthesised from pWT121/FPV 411(R) would have a MW of 69,168 and that the products from pWT121/FPV 502(R) and pWT121/FPV 412(R) would be 26 amino acids smaller at the N-terminus. As the three products are in fact the same size, it seems probable that the primary gene product has been processed and a portion of the polypeptide removed. The same results were obtained when the products of these plasmids were examined in minicells (unpublished results). Clearly the mechanism of the processing and the possible involvement of the pre-HA sequence need further investigation.

Conclusions

We have demonstrated the feasibility of producing controlled amounts of influenza antigenic determinants by genetic engineering. Obviously further analysis is necessary to characterise the protein product and a number of questions remain unanswered. First, for example, initiation of protein synthesis from the prokaryotic-like ribosome binding site on the HA will result in a polypeptide having a eukaryotic leader sequence. This sequence is thought to function in the transmembrane movement of the HA *in vivo*[26]; we may question whether this sequence is recognised by the regulatory systems for export in bacteria. Second, what effect do the $(T_{19}):(A_{19})$ region, resulting from the oligo(dT) primer and the poly(A)tail of the vRNA and situated between the AUG of *trpE* and the HA sequence, or the $(Phe)_6$ oligopeptide resulting from its translation, have on transcription and translation respectively? Homopolymeric nucleotide regions such as this are one of the signals implicated in transcription termination[27]; thus the $(T_{19}):(A_{19})$ region may have a polar effect on HA production. Similarly, the $(Phe)_6$ region may result in depletion of charged $tRNA_{Phe}$ and slow ribosome movement along the mRNA. Clearly examination of HA production by plasmids where this region has been removed is essential.

The absolute quantity of HA-like protein produced may also be estimated. The estimates based on immunological detection cannot be precise and are probably minimum values since if there are differences from the natural antigen it seems likely that the bacterial product would have fewer antigenic determinants or lower affinity for the antiserum. Based on our maximum yield of 60 ng per 50 μl bacterial lysate (Table 2) and a protein concentration of 160 μg ml^{-1} for the same lysate we arrive at a figure of 0.75% of total protein. By comparison, the quantities of induced proteins can be determined by densitometry from autoradiographs of gels containing proteins from induced and repressed cells. This shows that the 61,000 MW protein is 2–3% of total protein synthesis (unpublished results). Neither of these values was as high as expected. Hallewell and Emtage[28] have recently cloned an *E. coli HindIII* fragment containing the *trp* promoter and the complete *trpE* gene in pBR322; in induced conditions this plasmid was capable of specifying up to 30% of total protein as anthranilate synthetase[28]. Our values however are similar to those reported for the synthesis of ovalbumin-like proteins under the control of the *lac* promoter[29,30]; that is, the level of expression of the eukaryotic genes is only about 10% of the expected. Whether this is due to limiting amounts of some tRNA species as proposed by Fraser and Bruce[30] or to the type of transcription termination discussed above remains to be seen.

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LETTERS

A search for neutral hydrogen absorption in the double quasar 0957+561

C. L. Bennett, C. R. Lawrence & B. F. Burke

Department of Physics and Research Laboratory of Electronics,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

We report here the results of a search for neutral hydrogen absorption in the spectrum of 0957+561. This source consists of two quasars with similar optical spectra and Mg II absorption redshifts, and an extended region containing most of the radio flux. We place limits on the optical depth of intervening hydrogen of 0.02 and 0.23, depending on whether such hydrogen covers all of the source or only the compact components.

The quasars 0957+561 A, B were discovered by Porcas *et al.*[1] as two blue objects of nearly equal magnitude, separated by 6 arc s. Walsh *et al.*[2] have suggested that these two objects, having the same redshifts and essentially identical optical spectra, may be one source split into two images by a gravitational lens. High resolution maps at 6 cm by Roberts *et al.*[3] and Pooley *et al.*[4] do not disprove this model, but an equally likely and possibly simpler interpretation is that the source is a true double quasar, with one component presently ejecting relativistic plasma[3]. Spectroscopic observations by Weymann *et al.*[5] on the multiple mirror telescope give heliocentric vacuum Mg II absorption redshifts of 1.3911 for A and 1.3912 for B.

The radio absorption line observations reported here were conducted on the 300-ft telescope of the National Radio Astronomy Observatory in Green Bank, West Virginia, during a two week period in June 1979. The 515–740 MHz dual channel receiver, with a system temperature of about 130 K, was used to search the redshift range 1.388–1.394. Spectra were processed by the Mark III autocorrelator running in a parallel mode, with 192 channels devoted to each of the two independent receivers. A bandwidth of 2.5 MHz was used, yielding a channel spacing of ~3.5 km s^{-1}. The system was operated in the total power mode with off-source scans taken before and after on-source scans. The total on-source integration time was ~2h for each receiver, and the two receivers were averaged together in the data reduction. The resulting spectrum is shown in Fig. 1, and shows no evidence for neutral hydrogen absorption lines deeper than 0.02 K. The spectrometer system was checked by observing the H II region W43. The 222α hydrogen recombination line at 595.01 MHz was seen at the expected intensity.

Continuum antenna temperature measurements were made at 594 MHz with the same receiver; however, there is a confusing source, 4C+55.19, about 16 arc min south-east of the quasars (2/3 power point in the beam), of unknown 594 MHz flux. This source is clearly seen at the extreme edge of a full field VLA map of 0957±561 at 20 cm (Roberts, personal communication), but it is so far off-centre that the flux is unreliable. We estimate that the 594 MHz flux of 0957+561 is about 1 Jy, based on fluxes at 20 cm (Roberts, personal communication) and at 73 cm (Pooley *et al.*[4]). The corresponding 300 ft antenna temperature is ~0.9 K. The flux at 6 cm was measured with the 6–25 receiver, which has a system temperature of about 80 K, and found to be 230±20 mJy. Both measurements were made in July 1979.

The 6 cm VLA map of Roberts *et al.*[3] has an angular resolution of 0.8 arc s, and shows a pair of unresolved sources coincident with the optical quasars A and B (A being northernmost). In addition, there is a double, extended source 2–5 arc s north-east of A, and a probable extended source 5 arc s south-west of A, these sources being designated C, D, and E respectively by Roberts *et al.*[3] If the entire complex is covered by the absorber, the 0.02 K absorption limit and the 0.9 K estimated antenna temperature place an upper limit on the optical depth of $\tau \leqslant 0.02$. As we do not know where the optical lines are formed, however, this assumption may not be valid. A more conservative assumption is that only the compact sources A and B, which show almost identical Mg I, Mg II, and Fe II optical absorption lines, are covered. Of the total VLA flux of 220 mJy, source A contributed 36 mJy, and source B 30 mJy. Pooley *et al.*[4] report a 408 MHz flux of 120 mJy for A, and an upper limit of 50 mJy for B. Interpolating the fluxes between these two frequencies, we estimate that the combined 594 MHz flux of A and B lies between 100 and 150 mJy. In this case, our limit on the optical depth would be $\tau \leqslant 0.23$.

Both optical and radio absorption lines have been detected in the spectra of other compact sources. The Mg II and Fe II optical lines in the BL Lac object AO0235+164 and the quasar 1331+170 are the most similar to those in 0957+561; optical and radio results for these three are summarised in Table 1.

The equivalent widths of the optical lines in 0957+561 are intermediate between those of AO0235+164 and 1331–170. If the absorbing material covers only A and B, the resulting optical depth limit of $\tau \leqslant 0.23$ is consistent with the known optical depths of the H I absorption lines in the other two sources. Alternatively, if the absorber covers some or all of C, D, and E, the peak optical depth of 0.02 may imply a higher spin temperature or a lower density for the hydrogen in this absorber than for that in the other two absorption systems.

To place a limit on $n_{\rm H}$, the column density of hydrogen, we assume a rest frame line width of 10 km s^{-1}. With spin temperature $T_{\rm s}$, the limits are $(n_{\rm H}/T_{\rm s}) < 4 \times 10^{18}$ H cm^{-2} K^{-1} for $\tau < 0.23$, and $n_{\rm H}/T_{\rm s} < 4 \times 10^{17}$ H cm^{-2} K^{-1} for $\tau < 0.02$.

The optical lines are unresolved and highly saturated, so that only approximate information about column densities of Mg II can be extracted. From the equivalent widths of Weymann *et al.*[6] the doublet ratio of the Mg II lines is 0.92 for source A and 1.04

Table 1 Comparison of 0957+561 with similar absorption systems

Source		$z_{\rm abs}$	W_λ (rest frame) Mg II 2,796	Mg II 2,803	Fe II 2,586	Fe II 2,599	H I width (observed frame) (km s^{-1})	Optical depth at 21 cm	Refs
0957+561	A	1.391	2.0	2.2	1.0	1.2	—	<0.02*, <0.23†	1–5
	B	1.391	2.1	2.0	0.75	1.0			
AO0235+164		0.524	2.42	2.34	1.46	1.79	Total ≈ 50 Structure ⩾ 6	0.71 deepest line 0.53 2nd deepest	6–8
1331+170		1.776 1.785	1.30 2.15‡	2.16‡ 1.04	0.65 —	0.54 —	22.2	0.02*	9–11

* Absorber covering all of source.
† Absorber covering A and B components only.
‡ Lines blended.

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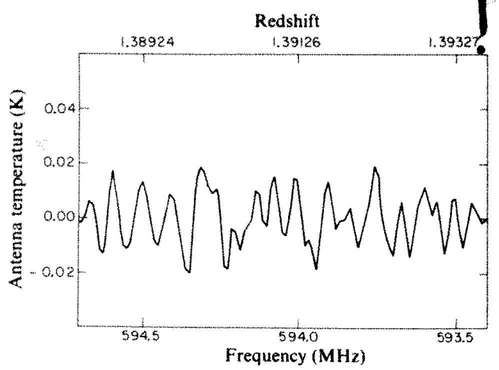

Fig. 1 Atenna temperature as a function of heliocentric redshift and frequency. The spectrum represents about two hours of on-source integration time, and has no absorption features greater than 0.02 K.

for B. Since the doublet ratio decreases from 2 to 1 as the ion density increases, we interpret the A value of 0.92 as a reflection of measurement uncertainties, and assign error limits to the B ratio of $1.04^{+0.08}_{-0.04}$. The resulting upper limit of 1.12 in the doublet ratio of Mg II corresponds to a lower limit on the column density of $n_{\mathrm{MgII}} > 10^{15}\ \mathrm{cm}^{-2}$. For $\tau < 0.23$ and $T_s > 10$ K this limit is consistent with cosmic abundances; for $\tau < 0.02$ spin temperature would have to be >100 K. These limits are, of course, strongly dependent on the equivalent widths of the unresolved optical absorption lines.

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Emission feature λ 3,470 of VV Puppis may be a cyclotron line

I. G. Mitrofanov

A. F. Ioffe Physics and Technology Institute, USSR Academy of Sciences, Leningrad 194021, USSR

The possibility that the unidentified emission feature λ 3,470 in the spectrum of VV Puppis is a cyclotron line is discussed and a theory to explain such an origin is presented.

VV Puppis displays a large linear and circular polarisation $P_{\mathrm{max}} \simeq q_{\mathrm{max}} \simeq 16\%$ (ref. 1) showing it to be an object of the AM Herculis type. These objects (AM Her, AN Ursae Majoris, VV Pup and 2A0311 − 227) are close binary systems containing an outflowing non-degenerate star and an accreting degenerate dwarf. Stars of the AM Her group have no erruptions and probably belong to the nova-like class. The polarisation of VV Pup light changes regularly with an orbital period of ~100 min. During ~60% of this period VV Pup usually increases its luminosity. Its magnitude varies from $m_{\mathrm{min}} = 14.4$–18 to $m_{\mathrm{min}} - \Delta m$ mag where $\Delta m = 0$–2.5. There seems to be no clear correlation between the m_{min} and Δm variations. The maximum positive circular polarisation q_{max} is observed in the phases of the orbital period when VV Pup is photometrically bright. In the phases of minimum light the circular polarisation is weaker and negative. The magnitude of q_{max} appears to change from one observation to another.

Although the VV Pup spectrum usually has strong H and He II emission lines, Angel *et al.*[2] have observed that this star sometimes has an unusual state of steady low luminosity with no strong H and He II emission. According to a more detailed description of these observations[3], in March and April 1977 VV Pup was in two different states. In March the magnitude in the minimum phases was small $m_{\mathrm{min}} \simeq 17.7$, brightening was large $\Delta m \simeq 2.5$ and the H and He II emission lines were strong. The average Hβ flux was about 1% of the continuum 3,500–6,000 Å flux. In April the minimum light was approximately the same, but brightening was small $\Delta m \leq 0.5$ and the spectra has no strong H, He or some other elements emission (Hβ flux did not exceed 0.02% of the 3,500–6,000 Å continuum). Nevertheless, a strong unidentified emission line near 3,470 Å was statistically significant[3] in both spectra obtained by a single-channel prism scanner of the Steward 2.3-m telescope (Fig. 1) and a Kitt Peak 4-m intensified image-dissector scanner.

The unidentified line cannot be an atomic line of some element, because other lines of such an element would also be observed. The feature has been observed using different equipment, so the coincidence of any systematic errors or 'glitches' seems unlikely. The feature corresponded to energy flux of about 3% of the 3,500–6,000 Å continuum, that is, it was very strong. When VV Pup was in the active state (March 1977), the emission feature λ3,470 was much smaller[3].

One possibility is that the strong emission feature λ3,470 is a cyclotron line. The magnetic field B corresponding to the wavelength of this line is equal to 3.1×10^8 G. The accuracy of this determination depends on the spectral width of the feature, the estimated error is $\Delta B = (\Delta\lambda/\lambda) \times B \approx 10^7$ G.

This magnitic field is likely to belong to the degenerate component of VV Pup. The presence of a magnetised degenerate dwarf in the AM Her system was predicted when the origin of the large circular and linear polarisation of AM Her light was discussed[4]. Two different sources of polarised light were proposed: (1) the optically thick cyclotron or synchrotron emitting accretion column[4–6] and (2) the photosphere of a magnetised degenerate dwarf[7]. In both cases the dwarf's magnetic field was estimated as $\gtrsim 10^8$ G. There is a good agreement between this estimation and the value 3.1×10^8 G following from our interpretation. A study of polarisation in the spectral region near λ3,470 would be required for a positive confirmation. Nevertheless, a short theoretical verification of this identification seems to be necessary.

The cyclotron emission, which is responsible for a cyclotron line, cools the emitting plasma very effectively. The necessary heating may arise from the accretion process when the gravitational energy of the accreted plasma is released near the surface of a compact star. The cyclotron emitting plasma accreted by a

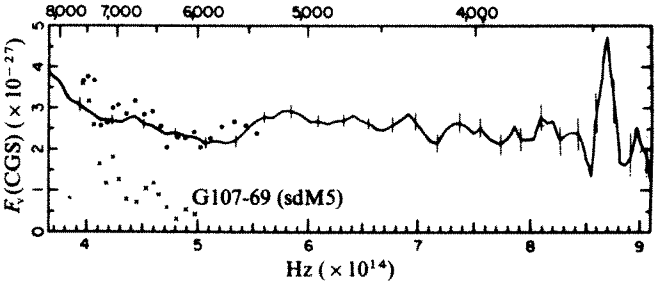

Fig. 1 The spectrum of VV Puppis obtained in April 1977 by Liebert *et al.*[3].

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magnetised neutron star was considered[8] for the conditions of an X-ray pulsar. The emission features corresponding to cyclotron harmonics were found to be present in the X-ray spectrum. Later the radiation of the accreting degenerate dwarf was considered in more detail[9-12], when the optical depth of the emitting region is sufficiently large with respect to the cyclotron absorption and the optical cyclotron continuum is radiated.

In April 1977, the VV Pup continuum source in 3,500–6,000 Å band was accepted to be the degenerate dwarf's surface with temperature 9,000 K (ref. 3). Therefore, at this low state the shock region was likely to be optically thin for cyclotron radiation and only narrow cyclotron line was radiated. The low luminosity states seem to correspond with the regime of weak accretion. The variations of the accretion result, for example, from the changes of the position of the outflowing star inside the dwarf's magnetosphere. The distance from VV Pup is about 80–150 pc. Using the composite energy distribution[3] one can estimate luminosities $L_{\mathrm{cont}} = (0.5\text{–}2)10^{30}$ erg s^{-1} and $L_{\mathrm{cyc}} = (2\text{–}6)10^{28}$ erg s^{-1} in the low state. It seems natural to assume that nearly all the gravitational energy released was radiated in the cyclotron line. To provide for the observed L_{cyc}, a weak accretion at a rate of $\dot{M} \simeq 3\times10^{11}\,\mathrm{g\,s^{-1}}\ (M_{\mathrm{dwarf}}/M_{\odot})\times(5\times10^{8}\,\mathrm{cm}/R_{\mathrm{dwarf}})$ is necessary (it is the low limit for accretion rate at low state). The radius of the Alfvèn surface seems to be much larger than the distance between the VV Pup's components. This means that the dwarf's magnetic field at every point determines the dynamics of the transferring plasma. Assuming the dipole field configuration, one can estimate the area S of the dwarf's surface where the accretion occurs. If the radius of the outflowing region on the non-degenerate component is of the order of its radius $\sim2\times10^{10}$ cm and the distance between the dwarf and the outflowing region is about 2.5×10^{10} cm, then $S \simeq 5\times10^{15}\,\mathrm{cm}^2$. The supposed cyclotron line should arise in the plasma heated by the standoff shock above this area. What are the conditions necessary for the line origin?

(i) The region of heated plasma should not be optically thick with respect to the cyclotron absorption. The corresponding optical depth in the direction perpendicular to the stellar surface is equal to[13]

$$\tau_{\mathrm{cyc}}(\nu) \simeq (2\pi)^{1/2}\langle nd\rangle\cdot\frac{e^2}{m_{\mathrm{e}}v_{\mathrm{e}}\nu_{\mathrm{B}}}\cdot\exp\left[-\left(\frac{c\Delta\nu}{\sqrt{2}v_{\mathrm{e}}\nu_{\mathrm{B}}}\right)^2\right] \tag{1}$$

where v_{e} is the thermal velocity of the electrons, e and m_{e} are their charge and mass, ν_{B} is the cyclotron frequency and $\Delta\nu$ is $\nu-\nu_{\mathrm{B}}$. The parameter $\langle nd\rangle$ is the mean product of the electron concentration n and the height d of the heated region. The value of v_{e} may be estimated from the condition that the observed mean linewidth is larger that Doppler broadening $\Delta\lambda/\lambda_{\mathrm{B}} \simeq 3\times10^{-2} \geqslant 2v_{\mathrm{e}}/c$. Hence $v_{\mathrm{e}} \leqslant 5\times10^{8}\,\mathrm{cm\,s^{-1}}$ and the electron temperature $T_{\mathrm{e}} \leqslant 10^6$ K. According to equation (1), the heated region is optically thin if $\langle nd\rangle \leqslant 10^{15}\,\mathrm{cm}^{-2}$. It is easy to check that for the cyclotron line all other absorption processes are not as essential as this one. For the equilibrium to set in between the pressure of the infalling plasma $\sim\dot{M}v_{\mathrm{ff}}/S$ and the thermal pressure inside the hot region $\sim2nkT$ it is necessary that $n = 10^{14}\,\mathrm{cm}^{-3}$ for $T = 10^6$ K. Therefore, d should be ~10 cm.

(ii) The plasma heated by the stand-off shock has to provide for the observed luminosity of the cyclotron line

$$L_{\mathrm{cyc}} \simeq \frac{B^2}{4\pi}c\sigma_{\mathrm{T}}\left(\frac{v_{\mathrm{e}}}{c}\right)^2 S\langle nd\rangle$$

$$\simeq 10^{14}\left(\frac{B}{3\times10^8\,\mathrm{G}}\right)^2\cdot\left(\frac{T}{10^6}\right)\cdot\left(\frac{S}{5\times10^{15}\,\mathrm{cm}^2}\right)\quad\mathrm{erg\,s^{-1}} \tag{2}$$

where σ_{T} is the Thomson cross-section of the electron. The value of L_{cyc} estimated from equation (2) agrees with the observed luminosity $\sim10^{29}\,\mathrm{erg\,s^{-1}}$ if $\langle nd\rangle$ is just the same, that is, $10^{15}\,\mathrm{cm}^{-2}$.

(iii) The magnitude of the parameter $\langle nd\rangle$ should be sufficiently large for the shock region to ensure the release of the total gravitational energy. For the Coulomb braking of protons by plasma with concentration $\sim10^{14}\,\mathrm{cm}^{-3}$ it is necessary that $\langle nd\rangle_{\mathrm{Cul}} \simeq 10^{19}\,\mathrm{cm}^{-2}\ (M_{\mathrm{dwarf}}/M_{\odot})^2\times(5\times10^8\,\mathrm{cm}/R_{\mathrm{dwarf}})^2$. This magnitude is too large compared to $10^{15}\,\mathrm{cm}^{-2}$ following from points (i) and (ii) above. However, one should take into account that the proton accretion flow is accompanied (and compensated) by the electron accretion flow. In this case, the braking appears to be much more effective due to the generation of sound and Langmuir oscillations and the interaction with the plasma turbulence[13].

Thus one can conclude that the observed[3] emission feature λ3,470 may be a cyclotron line arising due to the accretion at a rate $\sim3\times10^{11}\,\mathrm{g\,s^{-1}}$ in the heated region with $S \simeq 5\times10^{15}\,\mathrm{cm}^2$, $d \simeq 10$ cm, $n \simeq 10^{14}\,\mathrm{cm}^{-3}$, $T_{\mathrm{e}} \simeq 10^6$ K above the surface of the degenerated dwarf with $B \simeq 3.1\times10^8$ G.

Four implications follow from this conclusion:

(1) Some of the energy emitted in the cyclotron line should be absorbed by the surface of the degenerated dwarf giving rise to a hot spot. To find the exact relation between L_{spot} and L_{cyc} and the continuum spectrum of the hot spot one should solve the problem of radiation transport in the presence of a strong magnetic field and accretion heating. Nevertheless, it seems clear that all the released accretion energy is radiated in the cyclotron line and UV continuum when VV Pup is in the low accretion state (low steady luminosity).

(2) The increase of the accretion rate leads to increasing temperature and concentration in the heated region. It becomes optically thick for the cyclotron line and, as a result, the cyclotron continuum is emitted. The periodic brightening of VV Pup in the active state is thought to be associated with this continuum. Similarly, the increased accretion results in the strong H and He II emission. Thus one may predict an anticorrelation between the periodic brightening and the strong H and He II emission on the one hand, and the presence of the cyclotron line, on the other.

(3) It seems clear from the above discussion that the variation of the cyclotron line with the orbital period 100 min may be expected. It should be brighter in phases corresponding to the brightening in the active state. If there are two active magnetic poles[14] with slightly different magnetic fields, the frequency of the cyclotron line should vary with the phase.

(4) It may be possible to observe the cyclotron line in the AM Her and AN Ursae Majoris spectra when they have low luminosity. The cyclotron frequency is equal to $\nu_{\mathrm{B}} = 2.8\times10^{14}(B/10^8\,\mathrm{G})$ Hz ($\lambda_{\mathrm{B}} = 10{,}700\times(10^8\,\mathrm{G}/B)$ Å). It would also be of interest to search for a cyclotron line in the spectrum of the nova-like MV Lyra. For this star, a small variable linear and circular polarisation of 2% have been recorded[15]. In the spectral observations, the disappearance of the strong H and He II emission was discovered. It may be similar to the disappearance of strong H and He II emission from VV Pup. Thus MV Lyra can be considered as a candidate for the AM Her group.

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Hot hydrogen in the exosphere of Venus

T. E. Cravens, T. I. Gombosi* & A. F. Nagy

Space Physics Research Laboratory, Department of Atmospheric and Oceanic Science, The University of Michigan, 2455 Hayward,
Ann Arbor, Michigan 48109

The Ly α measurements of the hydrogen corona of Venus by Mariners 5 (ref. 1) and 10 (ref. 2) have been shown to be consistent with a two temperature component model. Bertaux *et al.*[3] have successfully fitted the Venera 9 exospheric Ly α data with an elevated (500 K) single temperature; however, they also indicated that their data are not inconsistent with a two component model. Various source mechanisms have been proposed to explain the 'hot' (~1,000 K), energetic component of the hydrogen corona. Here we use recent results from the Pioneer Venus Orbiter (PVO)[4] to establish the major sources of this hot hydrogen population.

The most viable of the suggested source mechanisms were reviewed and re-evaluated by Kumar *et al.*[5], who concluded that the exothermic reaction,

$$O^+ + H_2 \rightarrow OH^+ + H\ (<0.6\ \text{eV}) \qquad (1)$$

is the major source of the 'hot non-thermal' hydrogen. The subsequent recombination of OH^+ produces extremely energetic (~8 eV) hydrogen which makes only an insignificant contribution to the observed hot hydrogen, although it is the major contributor to the non-thermal escape flux.

Kumar *et al.*[5] also indicate that the reaction

$$CO_2^+ + H_2 \rightarrow CO_2H^+ + H\ (<1.17\ \text{eV}) \qquad (2)$$

can also make a modest contribution to the non-thermal population. Sze and McElroy[6] considered the charge exchange reaction:

$$H^+_{\text{hot}} + O \rightarrow O^+ + H_{\text{hot}} \qquad (3)$$

and Kumar *et al.*[5], Chamberlain[7] and Stewart[8] considered:

$$H^+_{\text{hot}} + H \rightarrow H^+ + H_{\text{hot}} \qquad (4)$$

where the hot hydrogen ion could be a solar wind ion or a hot ionospheric ion. These two charge exchange reactions were found to be unimportant for the hot hydrogen population, although reaction (4) was found to contribute to the non-thermal escape flux.

The very comprehensive ionospheric data base, which is now becoming available from the instruments on board the PVO[4] provides a new opportunity for a re-evaluation of the various proposed source mechanisms for the hot hydrogen corona. This letter has two purposes: first, to point out that reactions (3) and (4), as well as (1), are, in light of the PVO results, important sources of hot H from the nightside. Previous studies have never really explained why there was almost as much hot hydrogen observed by Mariner 5 (ref. 1) on the nightside of Venus as there is on the dayside. Ferrin[9] indicated that horizontal transport of hot hydrogen from the dayside might contribute, but he was puzzled by the fact that the nightside non-thermal H seems to be even hotter than the dayside one (1,500 K on the nightside and 1,025 K on the dayside according to Anderson[1]). We will show that the relatively large H^+ and O^+ densities[10] and high ion temperatures[11] (~5,000 K) observed by PVO on the nightside enhance the importance of reactions (3) and (4). Second, we re-evaluate the sources of hot H on the dayside of Venus, in light of the PVO results and conclude that the sources previously proposed are adequate.

Kumar *et al.*[5] calculated column production rates for various potential sources of hot hydrogen to assess their relative importance. To evaluate the hot hydrogen density at the exobase one must take into account collisions of the hydrogen atoms with the cold background gas. Ferrin[9] accomplished this by using Monte Carlo calculations and a multistream approach. He showed, by assuming a hard sphere collision model, that the inclusion of such collisions and the subsequent energy cascading results in significant enhancements of the calculated hot hydrogen densities. In the present work, we used a two stream approach, employed widely in radiative transfer[12] and photoelectron calculations[13], to evaluate the hot hydrogen fluxes and densities on Venus. The equations solved are:

$$\begin{aligned} \langle\cos\theta\rangle \frac{d\phi^+(E,z)}{dz} &= -\sum_i n_i\sigma_i\phi^+(E,z) + \frac{P}{2} + P^+ \\ -\langle\cos\theta\rangle \frac{d\phi^-(E,z)}{dz} &= -\sum_i n_i\sigma_i\phi^-(E,z) + \frac{P}{2} + P^- \end{aligned} \qquad (5)$$

where $\phi^+(E,z)$ is the upward directed hot hydrogen flux; $\phi^-(E,z)$ the downward directed hot hydrogen flux; $n_i(z)$ the i-th scattering atmospheric constituent; z the altitude; σ_i the total scattering cross-section for species i; $\langle\cos\theta\rangle$ the mean of the cosine of the angle of the hydrogen velocity vector with respect to the vertical (assumed to be 0.5, corresponding to an isotropic distribution); P the hot hydrogen production rate due to the various sources under consideration (assumed isotropic); and $P^\pm$ the 'production' rates at a given energy due to cascading from collisions at higher energies.

The energy distribution function, $f(E,z)$, can be determined from the calculated flux values, through the following relationship:

$$f(E,z) = \{\phi^+(E,z) + \phi^-(E,z)\}/v(E) \qquad (6)$$

where $v(E)$ is the hydrogen velocity corresponding to energy E.

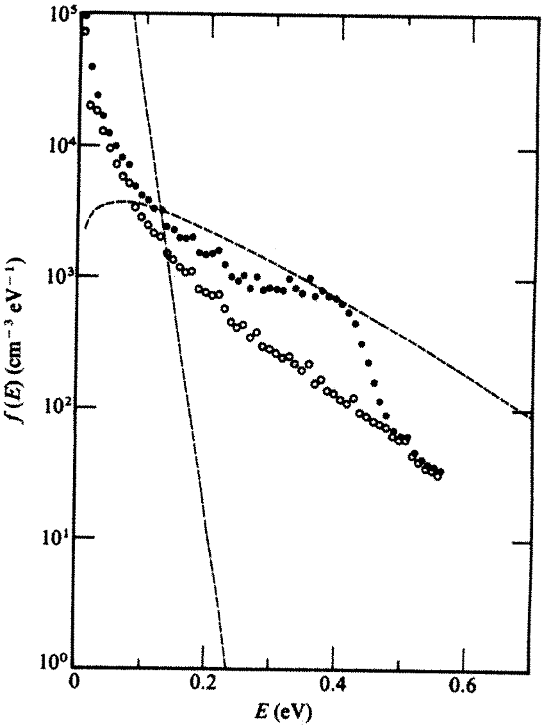

Fig. 1 The total calculated nightside density distribution function (●) is shown. ○, The distribution function including only the charge exchange reactions (3) and (4). The dashed lines represent the two component distribution functions deduced by Anderson[1] ($n = 2\times10^5$ cm^{-3} and $T = 150$ K for the cold and $n = 1\times10^3$ cm^{-3} and $T = 1,500$ K for the hot component).

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* Permanent address: Central Research Institute for Physics, Budapest, Hungary.

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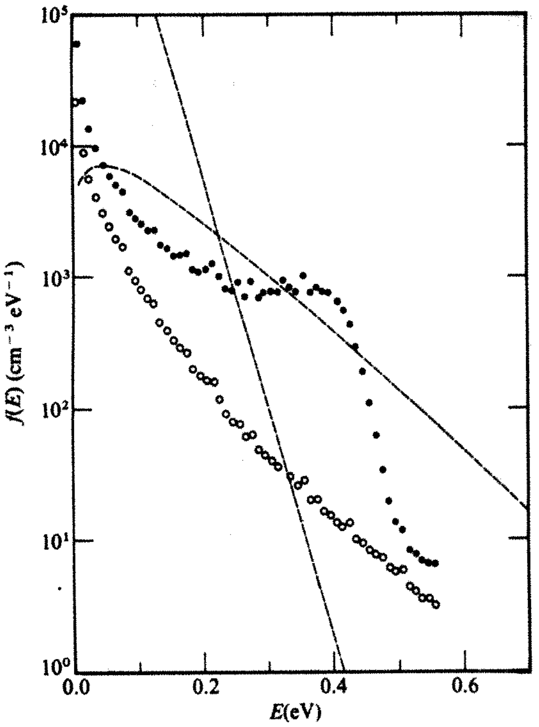

Fig. 2 The total calculated dayside density distribution function (●) is shown. ○, The distribution function including only the charge exchange reactions (3) and (4). The dashed lines represent the two component distribution functions deduced by Anderson[1] ($n = 2 \times 10^5$ cm^{-3} and $T = 275$ K for the cold and $n = 1.3 \times 10^3$ cm^{-3} and $T = 1{,}025$ K for the hot component).

The hot hydrogen atoms were assumed to be 'lost' either by escape at the topside or through a series of energy losses due to elastic hard sphere collisions[14] with the background gases (CO_2, O, H), eventually becoming part of the cold hydrogen population. We have neglected the small but finite probability of energy gain through these collisions. The average energy losses in backward and forward elastic scattering events are[14]

$$\overline{\Delta E_b} = \frac{3mM}{(m+M)^2} E_{in} \quad \text{and} \quad \overline{\Delta E_f} = \frac{mM}{(m+M)^2} E_{in}$$

respectively and where m is the mass of an H atom, M is the mass of the target and E_{in} is the initial H atom energy.

The appropriate neutral gas and ion densities as well as the ion temperatures were taken from Pioneer Venus data[10,11,15], except for the neutral atomic hydrogen densities, for which the Mariner 5 estimate of Anderson[1] was used, and molecular hydrogen, for which the estimate of Kumar and Hunten[16] was used. The adopted values of the dayside Pioneer Venus data correspond to zenith angles of about 70°, while the nightside data correspond to zenith angles of about 140°. The nightside ionospheric data were taken from the outbound segment of Orbit 59. The nightside ionosphere has been found to be extremely variable from orbit to orbit; therefore, the results of these calculations should only be considered as 'representative'.

The energy grid used in the two stream calculations was 0.01 eV and the altitude grid size was variable ranging from 0.2 km below 130 km up to 5 km above 250 km. The upper boundary was at 600 km. To approximate the escaping and ballistic components of the flux, the downward directed flux, ϕ^-, at the upper boundary, was assumed to be equal to the integral of the downward production above this altitude for energies greater than the escape energy (0.56 eV) and equal to the upward flux for lower energies. Although the upper boundary was set at 600 km, scattering by the background gas is not very important above the exobase level which is at about 170 km on the dayside and at about 145 km on the nightside. Consequently, above the exobase (or critical) level the distribution functions calculated using equations (5) and (6) remain almost constant with increasing altitude, because equation (5) only takes into account 'collisional' effects. What we obtain using equation (5) are distribution functions at the exobase level. We assumed that the H atoms from reactions (1) and (2) are produced with less energy than the exothermicities of the reactions would indicate, to take into account some degree of vibrational and/or rotational excitation of the product ions. H atoms from reactions (1) and (2) were assumed to be created with a gaussian distribution; the central energies were taken to be 0.4 eV and 0.8 eV respectively, with an assumed halfwidth of 0.03 eV. The hot H atom densities calculated including the effects of scattering (and cascading) by the background gas are about a factor of three larger than those calculated assuming no scattering and cascading. This is in line with the enhancement values found by Ferrin[9].

Anderson[1] found that he could fit the hot component of Mariner 5 nightside Ly α data by assuming a maxwellian distribution with an exobase density and temperature of 1×10^3 cm^{-3} and 1,500 K respectively. The energy distribution of the total hot hydrogen densities calculated for night-time conditions are shown in Fig. 1; for comparison the contribution due only to reactions (3) and (4) is shown with open circles, and the 'hot' and 'cold' maxwellian components deduced by Anderson[1] are indicated by dashed lines. These calculations indicate that the charge exchange reactions (3) and (4) are the source of about 50% and reaction (1) is the source of the other 50% of the night-time 'hot' hydrogen densities. Overall the calculated distribution function agrees reasonably well both in magnitude and shape with the deduced values in the energy range of 0.12 eV (the crossover point with the cold component) and 0.56 eV (the escape energy). The fact that the calculated distribution is not exactly maxwellian in shape is not of special concern, because the actually observed limb intensities do not uniquely require such a distribution.

The calculated dayside exobase distribution function is shown in Fig. 2 along with Anderson's dayside hot and cold maxwellian distributions. The overall hot density is about the same as the

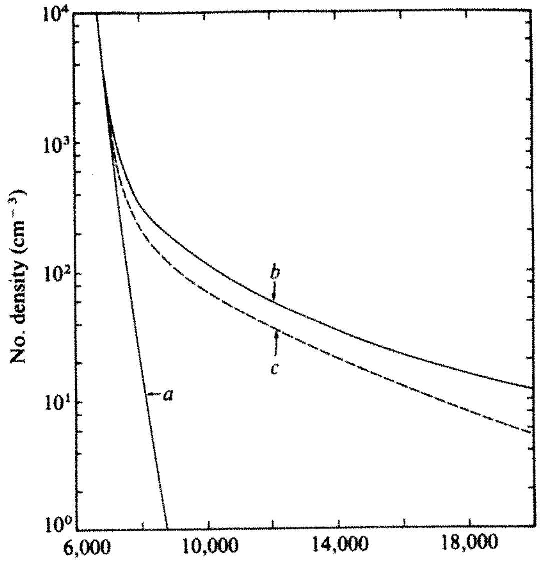

Fig. 3 H number densities for the nightside are shown as functions of the distance from the centre of the planet. a, Corresponds to the cold maxwellian of Fig. 1; b, is for both the cold and hot maxwellians of Fig. 1; and c, is for the cold maxwellian plus the non-maxwellian of Fig. 1.

maxwellian for energies greater than about 0.22 eV and less than 0.57 eV. The charge exchange sources contribute very little to the total hot density on the dayside. The $O^+ + H_2$ reaction is the dominant source of the nonthermal H.

Given a distribution function at the exobase level one can find the corresponding exospheric density distribution of H as a function of altitude by using Liouville's theorem[17]. We calculated the exospheric density distributions, using the distribution functions shown in Figs 1 and 2 and assuming (1) that the distribution functions are isotropic and (2) that there is spherical symmetry. Only ballistic and outgoing (escaping) hyperbolic orbits were included in our calculations. This should be a fairly good assumption because, for Venus, satellite particles will be rapidly removed by charge exchange with energetic solar wind or ionosheath protons. The calculated exospheric densities for the nightside are shown in Fig. 3. The total hydrogen altitude distribution (cold and hot maxwellians), has almost the same shape as density distribution *c* (the cold maxwellian plus our calculated nightside distribution), although the absolute magnitude is somewhat larger. We also calculated the total exospheric altitude distributions for the dayside but do not show them here because of space limitations and because the agreement between our calculated altitude distribution and the one deduced by Anderson[1] agree even better (~20%) than shown for the nightside case.

Note that, in comparing the results of these calculations with the Mariner 5 data, the atmospheric and ionospheric conditions were different during the Mariner 5 encounter and the Pioneer Venus observations and furthermore, the ionospheric densities are highly variable and only model values of H_2 are available. The calculations presented here indicate, within the limitations just mentioned, that reactions (1), (3) and (4) are the major sources of the 'hot' component of Venus hydrogen corona. On the nightside the charge exchange reactions are important because the ion temperatures are high and the H^+ densities are greater than on the dayside. There are preliminary indications, from the Pioneer Venus ion mass spectrometer results[10], that there is a significant night-time hydrogen bulge; if the night-time 'cold' hydrogen densities are greater than the values assumed in these calculations, the importance of reaction (4) will increase further. Contrary to earlier suggestions[9], most or at least a significant fraction of the nightside hot hydrogen population is apparently produced locally.

As the Pioneer Venus Ly α intensity results become available, more quantitative comparisons can be carried out. The nightside Venus ionosphere is highly variable, with apparent time scales of the same order as the hot hydrogen lifetime (~10^3 s) and therefore, orbit to orbit comparisons between the measured Ly α intensities and ionospheric parameters can be used to check the validity of the basic mechanisms suggested in this work.

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A weak interaction model for Io and the jovian magnetosphere

W.-H. Ip & W. I. Axford

Max-Planck-Institut für Aeronomie, D-3411 Katlenburg-Lindau 3, FRG

Four features of the interaction between the satellite Io and the surrounding jovian magnetosphere place constraints on the atmosphere of the satellite and on the nature of the interaction: (1) the atmosphere must be at least partly an exosphere so that sodium and other non-volatile atoms sputtered from the surface of the satellite could escape[1,2]; (2) the atmosphere should be dense enough for a detectable ionosphere to form showing a leading–trailing asymmetry[2,3]; (3) the atmosphere should provide an electrically conductive path to produce the electric currents which eventually cause the Io-related decametric radio emissions[4,5]; (4) the conductivity of the ionosphere should not be large enough to short out the electric field seen by the satellite, as pronounced absorption of energetic particles is observed[6–8]. Before the Pioneer 10 encounter it was pointed out[9] that erosion by the co-rotating magnetosphere caused atmospheres on the galilean satellites with surface pressures in the range 10^{-5}–10^{-9} mbar, if they exist, to be stable only if they are continuously replenished and therefore likely to be associated with "venting or the presence of non-hydrous ices on the surface". Alternatively, if the surface is covered with non-volatiles and/or water ice, the atmospheric pressure would be very low and controlled by sputtering due to the impact of magnetospheric plasma and energetic particles on the surface[9]. In any case, the interaction with the magnetosphere is likely to be 'weak' as it is for Mercury[10], that is, the magnetic field and plasma sweep into and past the satellite being affected mainly by the additional inertia of newly formed ions (provided the satellite is non-conducting and non-magnetic). We substantiate these arguments here using the recent Voyager observations, and suggest that the interaction between Io and the magnetosphere can be understood if the atmosphere of Io were largely exospheric in nature and controlled by the vapour pressure of SO_2 ice which covers the surface.

The discovery of volcanic activity on Io by Voyager 1 (ref. 11) confirms that venting of gas from beneath the surface must be an important source of gas in the atmosphere. By analogy with the gaseous products of terrestrial volcanoes (ignoring the H_2O and N_2 which probably have an external origin) one might expect CO_2 and SO_2 to predominate together with smaller amounts of SO_3, H_2S, CO, A, He, H_2 and Cl_2 (ref. 12). The behaviour of such volatiles, if ejected from the volcanoes on Io, can be studied in terms of a vapour pressure versus temperature diagram as shown in Fig. 1. Evidently CO_2 and several other possible constituents such as CH_4, CO and N_2 have such high vapour pressures that they would tend to completely dominate the atmosphere if they were present in the volcanic ejecta in any significant amounts. However, Voyager 1 observations of energetic heavy ions in the jovian magnetosphere indicate that S and O ions are dominant and C and N ions (other than those of obviously solar origin) are essentially absent[8]. Furthermore, SO_2^+ ions and their dissociation products were detected by the plasma experiment[13] and an SO_2 absorption feature was identified on the surface of the satellite by the IR and radiometry experiments[14]. Accordingly, we conclude that the gaseous emission from the Io volcanoes is almost entirely SO_2 and that the other components commonly found in terrestrial volcanoes are absent or very depleted.

To understand how this would affect the atmosphere of Io we should consider the situation where the surface of the satellite is uniformly covered with SO_2 frost and the atmospheric pressure is determined locally by the surface temperature (the effects of

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pressure gradients in inducing lateral flows are neglected). Figure 2 shows that the pressure should vary from ~1.3 × 10^{-3} mbar on the equator at the subsolar point ($T = 140$ K) to an exospheric level ($<2 \times 10^{-8}$ mbar) at elongations exceeding 80° from the subsolar point ($T < 100$ K). This maximum pressure exceeds the upper limits obtained from occultation measurements[15] but as these only apply to the limb, a detectable atmosphere would not be expected. The major constituents of the atmosphere should be essentially frozen out on the dark side of the satellite and over the whole surface during eclipses. However, ^{40}Ar, He and H_2, all of which could be expected to be present in volcanic ejecta in small amounts, could in principle survive such low temperatures and would provide most of the atmosphere in such situations. The argon could conceivably condense very close to the poles of Io and consequently have a very low average vapour pressure. This is not possible for hydrogen and helium. The evaporative and magnetospheric losses of these constituents must be severe enough to prevent them from becoming dominant. In fact, the ionisation time scale in the hot dense plasma which constitutes the magnetospheric environment of Io is sufficiently short (less than the orbital/rotation period of the satellite) that there is little chance for these constituents to accumulate. Similarly, secondary products such as O_2, which could be produced as a result of photolysis[16] or the effects of energetic magnetospheric particles, would also be lost rapidly.

We now consider the interaction between the magnetospheric plasma and magnetic field and the atmosphere of Io and in particular the abovementioned constraints on the interaction. The approximately exospheric (column content $\leq 10^{15}$ cm^2) nature of the atmosphere shown in Fig. 2 allows for the direct escape of sputtered material over a large part of the surface. As pointed out by Johnson *et al.*[2] a relatively dense 'ionosphere' can be produced in such a rarified atmosphere if the ionisation is due to the presence of a flux of relatively soft (10–10^2 eV) electrons, which are known to exist in the magnetospheric plasma torus (refs 3, 18, and H. E. Johnson and W. I. A. unpublished data). However, much of the ionosphere may not be as significantly affected by recombination as assumed by these authors because the convective removal time is shorter than the dissociative recombination time except in regions where the plasma density is $\gg 10^5$ cm^{-3} or the atmosphere is sufficiently dense to prevent rapid motion of the plasma. In these circumstances, the plasma density is controlled by convection giving rise to the leading–trailing asymmetry observed at the limb[3]. Obviously, over the subsolar point where the atmospheric density is large, the structure of the ionosphere must be much more complex and depends on the orbital phase of Io.

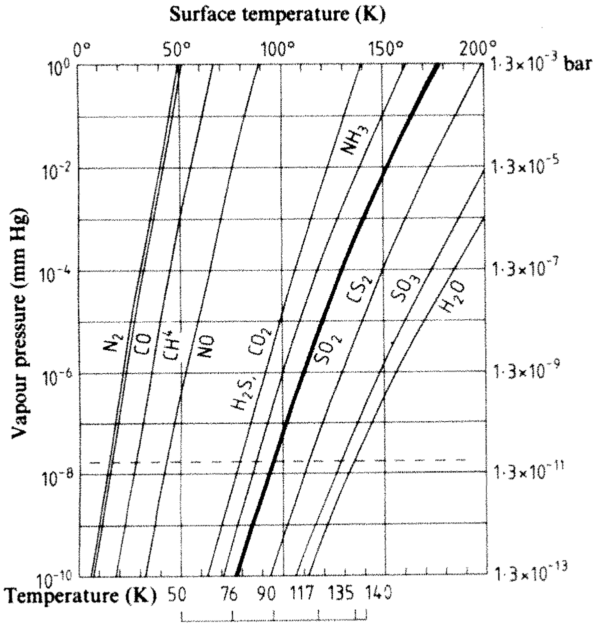

Fig. 1 The vapour pressures for various possible atmospheric constituents of Io as function of surface temperature. The curves are extrapolated from the data of refs 36, 37. The upper limit of the surface pressure determined by stellar occultation measurement is $\approx 10^{-4}$ mbar (ref. 15); and the broken line marks the exospheric limit of 3×10^{-8} mbar. The surface temperatures at various latitudes (θ) with a $(\cos \theta)^{1/4}$ dependence are marked in the bottom of the diagram.

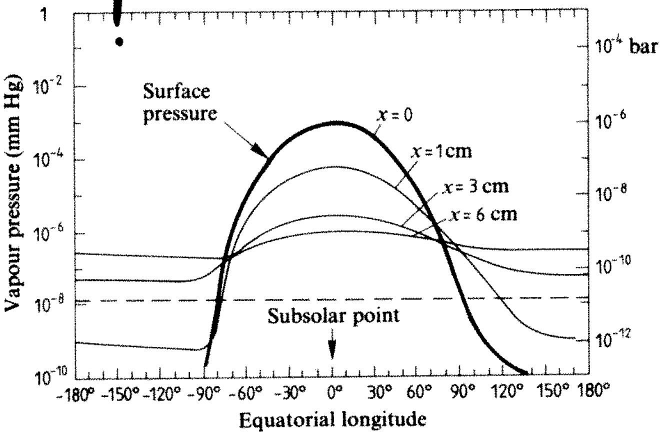

Fig. 2 The variations of SO_2 vapour pressure on Io as a function of longitude from the subsolar point. The curve for $x = 0$ is approximated from the numerical results of Sinton[38] and the curves for $x \neq 0$ are derived by assuming the surface material has thermal inertia 1.3×10^4 erg cm^{-1} s^{-1} K^{-1}, heat capacity 10^7 erg K^{-1} g^{-1}, a density of 0.1 g cm^{-3}, and thermal conductivity 1.7×10^2 erg cm^{-1} K^{-1} s^{-1}. Differing values of x correspond to differing thicknesses of a fine dust layer which could conceivably exist on parts of the surface at least.

As the atmosphere of Io is mainly exospheric the interaction with the magnetosphere is correspondingly weak except in the vicinity of the subsolar point where the surface pressure is relatively large. This implies that the incident magnetic field lines to a large extent cross the satellite relatively freely and the energetic particles they carry with them can suffer absorption on the surface as required by the observations[7,8]. However, the interaction should be capable of producing a fairly large electric current (~10^6–10^7 A) to account for the Io-related decametric radio emissions. That is, a drag must be exerted on the corotating plasma and the magnetic field correspondingly stressed, so that a disturbance in the form of an Alfvén wave propagates in both directions along the field lines towards the planet[4,5,19]. This could be to a large extent the result of ion–neutral collisions (Pedersen conductivity) in the dense atmosphere surrounding the subsolar point, in which case a very strong longitudinal control of the radio emissions might be expected. However, note that the effect of ion mass pick-up, which has previously been overlooked in this connection (although alluded to in ref. 9)), could make a significant if not dominant contribution in this respect.

The effects of ion mass pick-up in the presence of a magnetic field have been considered in contexts other than the Io–magnetosphere interaction, notably the interaction between the solar wind and comets[20,21], the ionospheres of Mars, Venus and Mercury[22–25] and the interstellar gas[26,27]. The processes involved can be understood using the mass and momentum conservation equations for the plasma which are good approximations in the circumstances of interest here:

$$\frac{\mathrm{d}n}{\mathrm{d}t} = q \tag{1}$$

$$\frac{\mathrm{d}}{\mathrm{d}t}(nmV) = nm\frac{\mathrm{d}\mathbf{V}}{\mathrm{d}t} + mq\mathbf{V} = \mathbf{j} \times \mathbf{B} \tag{2}$$

$$\mathbf{E} + (\mathbf{V} + \mathbf{B}) = \mathbf{j} \times \mathbf{B}/ne \tag{3}$$

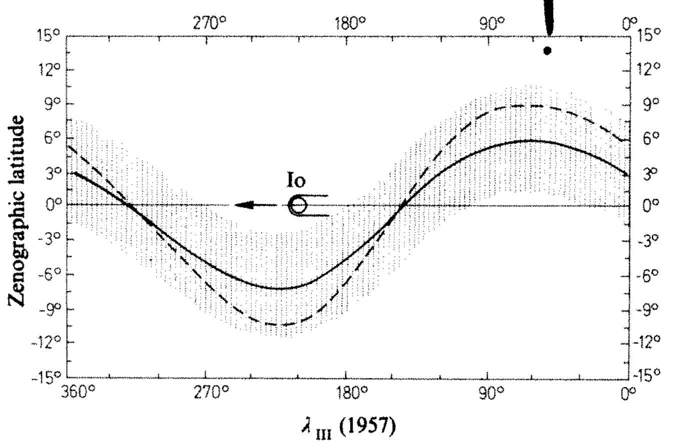

Fig. 3 Geometrical relationship between the corotating thermal plasma disk and the orbital position of Io. The shaded region represents the sulphur ion nebula with thickness equal to one jovian radius[32–34]. The solid curve indicates the central plane of the plasma disk, and the broken line the magnetic equator according to the Goddard Space Flight Center O_4 magnetic field model[35]. The direction of motion of Io in the rotating frame of the jovian magnetosphere and the convection pattern of the ionospheric plasma are also sketched. A current directed outward from Jupiter is generated in the extended atmosphere of Io to accelerate the newly created satellite ions into corotational motion. Io periodically crosses the plasma disk/magnetic equator at System III longitudes $\lambda_{\rm III} \approx 120°$ and 315°.

n is the plasma density, $\mathbf{V}$ is its velocity, m is the mean ion mass, q is the effective source strength due to ionisation of neutrals, $\mathbf{E}$ is the electric field, $\mathbf{e}$ the charge on an electron, and $\mathbf{j}$ and $\mathbf{B}$ are the electric current and magnetic induction respectively. As a first approximation, we neglect the effects of plasma pressure gradients and ion-neutral collisions and note that q must be proportional to the neutral density N regardless of the ionisation process. These equations, together with Maxwell's equations and suitable boundary conditions, determine the behaviour of the plasma. The two terms on the left of equation (2) can be regarded as sources of electric current, the first of which involves the deceleration and acceleration of the plasma (that is the polarisation current) and the second describes the effects of mass loading due to pick-up or loss of plasma by ionisation of neutrals and recombination or absorption, respectively. The latter term is negligible in most ionospheric situations but not in the case under consideration.

Taking $q = \alpha N$ with α and also $\mathbf{V}$ constant to a first approximation, we find from equation (1) that

$$\begin{aligned} n' &= (\alpha N_0 H/V)\exp(-z/H) \text{ upstream} \\ &= \alpha N_0 H/V(1-\exp(-z/H)) \text{ downstream} \end{aligned} \qquad (4)$$

where n' is the additional plasma density produced by ionisation of neutrals, z is the height above the surface of Io (for normal incidence), and N_0H is the column content of the atmosphere. These solutions are, of course, highly idealised but they demonstrate certain basic features of the interaction, notably the upstream–downstream asymmetry and the relationship between the scale heights of the ionosphere and neutral atmosphere. To produce the additional plasma density of $5\times10^3\ \text{cm}^{-3}$ observed on the upstream side of Io by Pioneer 10 (which, as it used a refraction technique, was unable to detect the uniform ambient magnetospheric plasma) α must be $2.5\times10^{-5}\ \text{s}^{-1}$ for $N_0H \approx 10^{15}\ \text{cm}^{-2}$.

Clearly it is not easy to predict the form of the electric current distribution resulting from such an interaction as it requires a knowledge of the precise form of the flow field as well as the sources and sinks of plasma and if full three-dimensional treatment is warranted. However, one can estimate the magnitude of a typical component of the current associated with mass pick-up (equation (2)) as simply

$$I = \dot{M}V/BL \qquad (5)$$

Here $\dot{M}$ is the total mass picked up by the magnetosphere and L is a length which is of the order of the diameter of Io. We estimate that if $\dot{M} \approx 2.5\times10^5\ \text{g s}^{-1}$ on the upstream side of Io then with $V = 57\ \text{km s}^{-1}$ and $L = 4{,}000$ km, the corresponding electric current induced is $I \approx 2\times10^6$ A. For the whole interaction, including the downstream region, the electric current may be significantly larger. The effective ionospheric conductance associated with ionisation of the exosphere is $\Sigma = I/EL \approx 5$ S and the energy dissipation is $IEL \approx 8\times10^{18}\ \text{erg s}^{-1}$. Note that the effects of the inertial term on the left hand side of equation (2) tend to reduce the current density on the upstream side of the satellite where the plasma decelerates by a factor of order $(1+X^2)^{1/2}$ where $X = \omega L'/V$, L' is a characteristic length, and ω is the gyrofrequency of the newly created ions in the ambient magnetic field. This reduction is significant on the upstream side as $L' \approx H$ and $X \approx 1$, but there is no diminution of the current overall as all the freshly produced ions must be accelerated eventually up to the corotational speed of the magnetosphere relative to Io. It can be seen that equation (5) also applies to the Io torus of more extensive structure.

All aspects of the interaction between Io and the jovian magnetosphere can, therefore, be accounted for if the atmosphere has the properties indicated in Fig. 2 and we suggest that ion mass pick-up is an important ingredient of this interaction. These arguments lead to some interesting conclusions on the variation of the nature of the interaction with both the jovian longitude and the Io phase. As the dense regions of the atmosphere influence the interaction the phase of Io in its orbit must be important as, for example, the subsolar point is on the leading–trailing side of the satellite at western–eastern elongations respectively. Also the fact that the jovian plasma sheet is not coplanar with the orbit of Io (see Fig. 3) should lead to a jovian longitudinal control of the currents produced by the interaction if the total ionisation rate is the determining factor and this is controlled by the density of the corotating magnetospheric plasma. Note that the plasma torus need not instantaneously coincide with the equilibrium position shown since it can oscillate in position along the magnetic field with a frequency $\sqrt{3}\Omega/2\pi$, where $2\pi/\Omega$ is the rotational period of Jupiter. This is not commensurate with the (relative) orbital period of Io and hence the addition of fresh plasma with random phase will tend to supress the oscillations. Nevertheless, the oscillatons should be completely absent only at longitudes $\lambda_{\rm III} \approx 120°$ and 350° where Io crosses the jovian magnetic equator.

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Note that several of our conclusions have been reached independently in papers submitted later than our original manuscripts. In particular, we refer to the discovery of SO_2 gas on Io by Pearl *et al.*[28] and the identification of SO_2 frost on the surface by Fanale *et al.*[29]. Smith *et al.*[30], Pearl *et al.*[28] and Kumar[31] have also drawn attention to the fact that the atmosphere of Io should be spatially inhomogeneous.

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Accretion of nitrogen during the growth of planets

John F. Kerridge

Institute of Geophysics, University of California, Los Angeles,
California 90024

The ^{15}N/^{14}N ratio measured in nitrogen of the terrestrial atmosphere is, or was, apparently widespread in the inner Solar System. Such an observation might suggest that this value (3.61×10^{-3}) represents that of proto-solar material. However, I argue here that the original ratio was at least 20% lower, the presently observed value having resulted from significant isotopic fractionation, presumably caused by nebular condensation processes.

First, I will summarise what we know about ^{15}N/^{14}N of inner Solar System objects, expressing these values as per cent deviations from the terrestrial atmospheric value. The Moon is highly depleted in volatiles, and identification of indigenous lunar N, free from either terrestrial contamination or cosmic ray spallation products, has consequently been very difficult. The best estimate for the lunar ^{15}N/^{14}N ratio is between +1 and +4% relative to air[1].

The martian atmosphere is highly enriched in ^{15}N, about +62% relative to air[2]. However, it has been argued that this value has resulted from selective escape of ^{14}N from the martian upper atmosphere[2]. When allowance is made for this fractionation, a starting composition close to the terrestrial value is derived[2]. Because of the model dependence of the fractionation calculations, it is not possible to assign realistic uncertainty limits to the initial ^{15}N/^{14}N ratio. A large uncertainty is also associated with the ^{15}N/^{14}N ratio found for the atmosphere of Venus. The value measured by Pioneer Venus is within 20% of that of terrestrial air[3].

Different groups of stony meteorites have significantly different N isotopic compositions[4,5], although the actual nature of the relationship between ^{15}N/^{14}N ratio and chemical–petrographic classification is unclear. Referring to Fig. 1 of ref. 5, ordinary chondrites and carbonaceous chondrites of petrologic types 3 and 4 lie close to the terrestrial value (−0.3 to +2.0% and −4.3 to +0.2%, respectively). Carbonaceous chondrites of petrologic types 1 and 2 are enriched in ^{15}N by up to 4.6% (with one large anomaly, Renazzo, at +17.2%), the degree of enrichment roughly correlating with increasing N content[5]. Enstatite chondrites, on the other hand, are depleted in ^{15}N (to −4.3%), with a rough tendency for ^{15}N/^{14}N to decrease with increasing N content[5]. Whatever the cause(s) of these isotopic patterns, the trend of the meteoritic data suggests that the present compositions reflect fractionations, in different directions, of a starting composition close to that of the terrestrial atmosphere. Apparently, formation of organic matter in carbonaceous chondrites and inorganic nitrides in enstatite chondrites favoured heavy and light isotopes, respectively.

This picture of isotopic uniformity in the inner Solar System is disturbed when we turn to the only other repository of N analysed so far: the solar wind, believed to represent the composition of the solar convective zone[6]. These analyses have been made using solar wind ions implanted in the lunar surface during the past 4 Gyr, and have revealed that ^{15}N/^{14}N in the Sun has increased substantially over the lifetime of the Moon[7]. The actual magnitude of the change is still not established precisely but is known to be at least 30%, from −19% (ref. 8) to +11% (ref. 1) relative to air. Whatever the cause of this change, possibly nuclear reactions in the solar convective zone[7], it seems clear that the solar atmosphere must have had an initial ^{15}N/^{14}N ratio at least 19% lower than terrestrial air. It is therefore difficult to avoid the conclusion that this lower value must also have characterised the proto-solar nebula. This in turn leads to the conclusion that the value observed or inferred for the Earth and other planetary objects must represent the result of significant isotopic fractionation.

It is unlikely that we can specify, at this time, how such fractionation occurred. The fact that its magnitude was apparently independent of either the size of the planetary object (radii varying from some tens of kilometres[9] to more than 6,000 km) or its degree of volatile depletion (from about 10^{-7} to 10^{-3} g N per g) suggests that N, and presumably other volatiles, were accreted by early forming planets, and planetesimals, in the form of a solid component of volatile-rich condensate. Isotopic fractionation apparently took place between this component and the reservoir from which it condensed, rather than during accretion. The magnitude and sign of the fractionation suggest gas–solid equilibration at very low temperature rather than kinetic effects or isotopically selective loss of gas.

Because neither the nature of the N-rich condensate nor its temperature of formation is known, it is not possible to determine whether the fractionation factor associated with the condensation reaction(s) is adequate to provide the degree of fractionation implied here. Calculated fractionation factors[10] for isotopic exchange between simple N compounds imply an equilibration temperature significantly below 273 K. Equilibration at such low temperatures in the nebula seems intuitively unlikely but similar temperatures are suggested by the enrichment of the Earth and carbonaceous chondrites in deuterium, relative to its abundance in the primitive solar nebula[11–13].

The condensation/accretion scenario suggested here is qualitatively consistent with the model of Anders and Owen[14], which proposes acquisition of the Earth's volatiles via a veneer of composition similar to that of carbonaceous chondrites of petrologic type 3, which also provide a reasonable match in ^{15}N/^{14}N ratios[5]. However, the magnitude of the fractionation suggested here, between the volatile-carrier and its parental reservoir, implies a temperature of formation significantly lower than the 400–450 K inferred by Anders and Owen for carbonaceous chondrite material[14].

The hypothesis presented here is potentially testable as it leads to three observational predictions. First, the ^{15}N/^{14}N ratio in Jupiter, which should directly reflect that of proto-solar material, will be found to be at least 19% lower than terrestrial air. Second, the ^{15}N/^{14}N ratio in the present solar wind will be found to be about 11% greater than in air (more if we do not measure it soon). Third, isotopic fractionation of N during condensation may have been accompanied by similar fractionation of any other volatile elements condensing at the same time. If such elements, or the appropriate fractions of them, can be isolated for analysis, similar heavy isotope enrichments may be detected. In fact, the deuterium mentioned above may constitute such evidence; however, the relationship between the provenance of nitrogen and that of hydrogen in early Solar System objects remains a topic for further study.

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Dynamical test of the law of gravitation

Hiromasa Hirakawa, Kimio Tsubono & Katsunobu Oide

Department of Physics, The University of Tokyo, Bunkyo, Tokyo 113, Japan

Although well established in the realm of celestial mechanics, gravitation between two masses has not yet been thoroughly examined in a laboratory. There have been several tests of the interaction since the Cavendish's celebrated experiment[1–6], most of them measuring the static attraction between two spherical masses at a distance R less than 0.8 m. The gravitational interaction is weak and, in contrast with the electromagnetic interaction, there is no effective means to shield it. It has therefore proved difficult to increase the distance R over 1 m. Under the circumstances several authors[7–9] have noted that the existing experimental data do not exclude a possible term which is added to the newtonian $1/R$ potential. Meanwhile, on the grounds of elementary particle physics, a number of theoretical papers[7,10–14] have been published predicting additional terms which vanish rapidly at $R = \infty$. We now report a test of the law of gravitation in a range of R up to 4.2 m. The experiment is based on a low frequency dynamical quadrupole–quadrupole interaction between two masses, which was first observed by Sinsky and Weber[15,16]. Our result agrees with the inverse square law within the accuracy of the experiment.

A steel bar of length $b = 0.52$ m and mass $m = 44$ kg was rotated in a horizontal plane at frequency $f = 30.3$ Hz and the resulting dynamic gravitational field was measured by a mass quadrupole antenna[17] resonating at $2f = 60.5$ Hz (Fig. 1). The antenna is a 1,400-kg aluminium square plate with cuts on each side, contained in a vacuum tank of a constant temperature, $T = 308$ K. The oscillation of the antenna was sensed by an electrostatic transducer of field strength 4.6×10^6 V m^{-1} mounted inside one of the cuts. The quality factor Q of the unloaded antenna was 65,000 at 60.5 Hz. The resulting width of the resonance, 9.3×10^{-4} Hz, was inconveniently narrow for a long-term operation requiring a precise frequency tracking. Therefore we applied a cold damping[18] to the antenna, loading it with an artificial cold resistor[19], 6.3 MΩ at 3.0 K. This resulted in a reduced quality factor $Q' = 4{,}100$, a resonance width 1.5×10^{-2} Hz, and a noise temperature $T' = 22$ K of the antenna. With the rotor located at $R = 2.2$ m, the antenna oscillated with an amplitude $x = 1.9 \times 10^{-13}$ m in regard to the transducer displacement. This yielded a coherent 8.7×10^{-7} V (peak) transducer output, while the background noise, consisting mostly of the antenna brownian motion at $T' = 22$ K, was 1.2×10^{-8} V (r.m.s.).

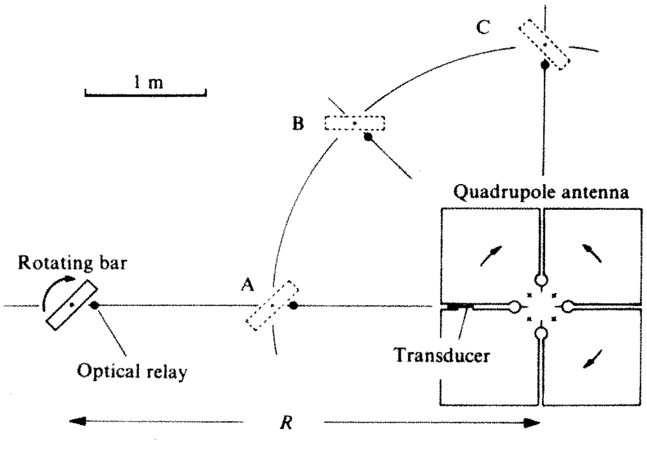

Fig. 1 Dynamic gravitational field of a 44-kg steel bar rotating at 30.3 Hz was measured by a 1,400-kg aluminium quadrupole antenna resonating at 60.5 Hz. Signals were obtained for a distance R up to 4.2 m.

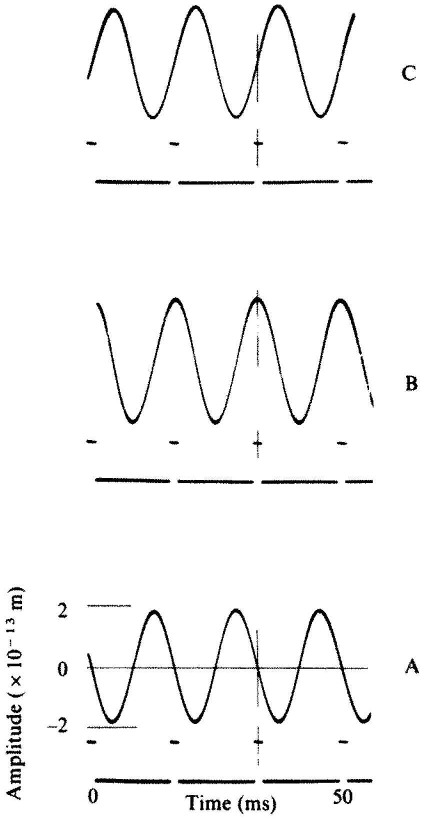

Fig. 2 Oscillation of the antenna observed on a cathode-ray tube with the rotor located at A, B and C of Fig. 1 respectively. Distance $R = 2.2$ m. The timing pulses obtained from an optical relay indicate the rotation angle of the bar. Note the difference in the phase of oscillation in three pictures. The signal at B is slightly larger than the others, showing the effect of the higher mass multiple moments of the antenna. With an increasing distance R, the ratio of the signal B to signal A (=C) tends to 16/19.

To eliminate a possible interaction of the antenna with coherent noises in the background, we rotated the bar in a steel vacuum tank using a plastic chain and two sprocket wheels of a teeth ratio 8/5. The larger wheel was mounted on a common shaft of a 400-W induction motor and a phase-locking 200-W synchronous motor, the latter being driven by a 37.8-Hz three-phase power supply connected to a quartz frequency synthesiser. In spite of these measures, we noted a coherent magnetic field around the tank, 10^{-4} G at 60.5 Hz and a smaller amount at 30.3 Hz, presumably originating from a geomagnetic or remanent magnetisation of the rotating steel bar. We checked, however, that a magnetic field, 5 G at 60.5 Hz, more than 90 db above the background was needed to produce a

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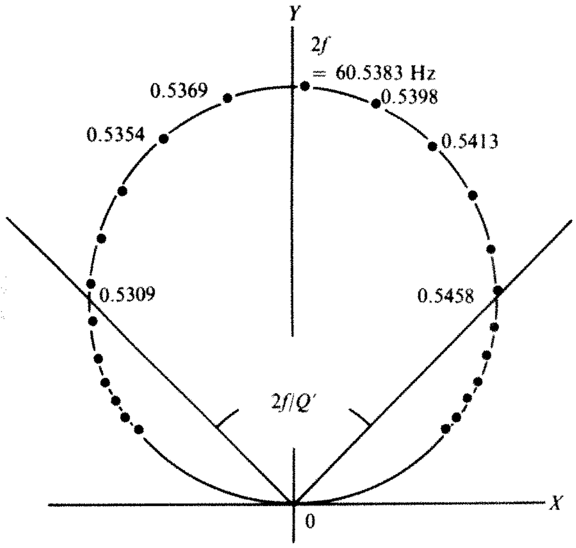

Fig. 3 · Characteristics of the antenna resonance at 60.5 Hz. The amplitude and phase of the forced vibration of the antenna is given as a function of the frequency $2f$ of the oscillating field. Quality factor of the antenna was reduced to $Q' = 4{,}100$ by application of a cold damping.

spurious antenna output of the same size as the observed signal. The rotor also caused vibration of the laboratory floor at 60.5 Hz. Two decks of pneumatic isolation pads were inserted below the rotor tank. They were more than enough to eliminate the contamination of the signal through this channel. In fact no change of signal was noted when one of the decks was removed.

The behaviour of the mass quadrupole antenna in the gravitational field of the rotating bar obeys an equation of forced motion of a damped linear oscillator quite well. The amplitude of vibration is given by

$$x = \frac{\gamma G a Q' m b^2}{R^5 f^2}$$

where a is the size of the antenna. γ is a numerical coefficient which depends on the geometry of experiment including the shape, the size, and the relative orientation of the masses. Figure 2 shows the antenna output signal obtained with the rotor at three locations around the antenna. The timing signal indicating the angle of the bar is also shown. The characteristics of the antenna resonance are given by a circular locus of Fig. 3 showing the observed amplitude and phase of vibration as a function of the frequency $2f$ of the oscillating field. The signal decreases with increasing distance R as $1/R^5$. However the signal-to-noise ratio can be improved substantially by means of Fourier integration[20] of the antenna output with the rotor timing signal as a reference. Thus we obtained a signal at $R = 4.2$ m, 30 db above the brownian motion, with an integration time of 4 h. Figure 4 compares the observed signal and the expected signal based on the inverse square law, with the distance R as a parameter. This result confirms Newton's law of gravitation within the range and accuracy, ±3%, of the experiment. For a parameter δ in an assumed form, $1/R^{1+\delta}$, of the potential, we have obtained 0.000 ± 0.053.

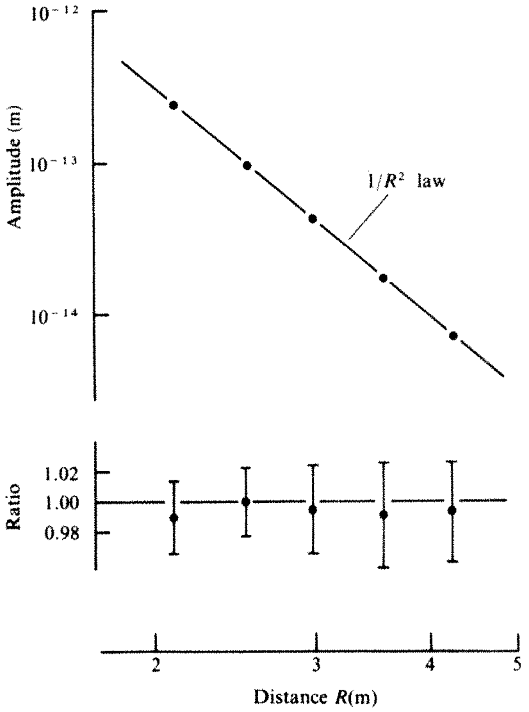

Fig. 4 The observed and calculated intensities of the signal and their ratio as a function of the distance R. The scale of the observed signal is normalised to the calculated signal at $R = 2.5$ m. The calculation is based on the inverse square law of gravitation. The effect of the higher multipole moments, the finite size of the rotor and the antenna, is taken into account by a numerical integration over the masses.

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Stress corrosion cracking of biotite and feldspar

R. L. Barnett & R. Kerrich

Department of Geology, University of Western Ontario, London, Ontario, Canada N6A 5B7

Pervasive intragranular cracking of biotite and feldspar has been observed in a naturally deformed granodiorite, where the leading edge of cracks are sites for specific reactions of parent grains to secondary mineral products. This textural feature provides evidence for an interrelationship of chemical reactions to slow crack propagation, and is considered to be a natural example of stress corrosion cracking. It is probable that the mineral reactions are catalysed by increase of the stress intensity at fracture tips, with reaction rates controlling propagation of the fractures.

Table 1 Chemical analyses of a biotite grain with cracks containing secondary minerals

	1		2	3	4	5	6	7	8
SiO_2	35.64	(0.22)	35.27	35.16	35.32	35.88	44.83	48.57	35.07
Al_2O_3	18.19	(0.18)	18.09	18.33	18.52	18.41	30.91	34.32	18.46
TiO_2	4.19	(0.14)	3.73	3.39	3.07	2.66	3.16	1.36	1.63
FeO	20.85	(0.31)	21.19	21.49	21.13	21.71	5.21	0.91	23.51
MgO	7.14	(0.45)	7.04	7.00	7.38	7.85	1.89	0.41	7.73
MnO	0.21	(0.07)	0.16	0.29	0.16	0.20	—	—	0.19
CaO	—		—	—	—	0.05	—	—	0.05
Na_2O	0.13	(0.04)	0.13	0.07	0.04	0.10	0.18	0.14	0.04
K_2O	9.50	(0.18)	9.76	9.63	9.72	9.47	9.63	8.75	9.21
	95.87	(0.54)	95.37	95.41	95.34	96.33	95.81	94.46	95.89
Si	5.437	(0.047)	5.428	5.419	5.426	5.461	6.067	6.400	5.409
Al(IV)	2.563	(0.047)	2.572	2.581	2.574	2.539	1.933	1.600	2.591
Σ	8.000		8.000	8.000	8.000	8.000	8.000	8.000	8.000
Al(VI)	0.707	(0.050)	0.709	0.747	0.779	0.764	2.996	3.729	0.765
Ti	0.481	(0.014)	0.432	0.393	0.355	0.304	0.322	0.135	0.189
Fe	2.660	(0.047)	2.727	2.770	2.715	2.764	0.590	0.100	3.033
Mg	1.622	(0.095)	1.615	1.608	1.690	1.781	0.381	0.081	1.777
Mn	0.031	(0.011)	0.021	0.038	0.021	0.021	—	—	0.025
Σ	5.501		5.504	5.556	5.558	5.638	4.289	4.044	5.786
Ca	—		—	—	—	0.008	—	—	0.008
Na	0.039	(0.013)	0.039	0.021	0.012	0.030	0.047	0.036	0.012
K	1.848	(0.032)	1.916	1.893	1.905	1.839	1.662	1.471	1.812
Σ	1.887		1.955	1.914	1.916	1.876	1.710	1.506	1.832

(1) Average of five analyses from the centre of a primary biotite grain. Figures in parentheses represent one standard deviation. (2) Biotite parent grain 200 μm from crack. (3) Biotite parent grain in pale domain 15 μm from crack. (4), (5) Biotite parent grain at leading edge of crack. (6), (7) Secondary high-Ti muscovite coexisting with secondary ilmenite in crack. (8) Secondary biotite recrystallised from parent at margin of grain.

Table 2 Chemical analyses of orthoclase and plagioclase grains with cracks containing secondary feldspar

	1		2	3	4	5	6	7	8	9	10	11
SiO_2	65.05	(0.38)	64.92	68.19	64.97	68.25	62.29	65.04	61.41	64.81	69.18	66.07
Al_2O_3	17.04	(0.31)	18.09	20.77	18.33	19.55	23.60	18.45	23.10	23.56	22.53	17.91
CaO	0.06	(0.03)	0.15	0.66	0.02	0.77	5.48	0.03	4.92	1.97	1.77	0.07
Na_2O	0.45	(0.03)	0.39	10.45	0.38	11.16	8.39	0.44	6.22	7.41	6.54	0.48
K_2O	16.43	(0.11)	16.32	0.37	16.58	0.14	0.59	16.64	2.51	2.15	1.38	15.98
Total	99.05	(0.66)	99.87	100.43	100.07	99.87	100.34	100.60	98.17	99.90	101.39	100.52
Si	12.152	(0.041)	12.020	11.785	12.020	11.883	11.031	11.977	11.074	11.398	11.820	12.113
Al	3.752	(0.056)	3.949	4.230	3.953	4.012	4.926	4.004	4.992	4.883	4.535	3.867
Na	0.164	(0.010)	0.141	3.836	0.137	4.105	2.879	0.156	2.211	2.527	2.168	0.172
Ca	0.014	(0.008)	0.031	0.121	0.004	0.145	1.039	0.004	0.965	0.371	0.324	0.016
K	3.917	(0.002)	3.855	0.082	3.914	0.031	0.133	3.910	0.586	0.484	0.301	3.738
Σ	20.000	(0.010)	19.996	20.055	20.027	20.176	20.008	20.051	19.828	19.664	19.148	19.906

(1) Orthoclase parent grain with fractures containing secondary fibrous orthoclase (2). Values in parentheses represent one standard deviation. (3) Albite parent grain with both secondary orthoclase (4) and albite (5) fracture fillings. (6) Oligoclase parent grain with secondary orthoclase (7) in fractures. (8) Parent oligoclase grain with abundant fine grained sericite, recrystallising to a less calic, sericite-free plagioclase at the leading edge of (10) and within fractures (9). Also present in the oligoclase parent grains are fractures containing secondary orthoclase (11).

The process of stress corrosion cracking has been reviewed by Anderson and Grew[1]. Slow crack growth at subcritical stress intensities by stress corrosion contrasts with fast irreversible propagation of 'Griffith cracks' above the critical stress value for brittle failure. Subcritical crack growth has been extensively studied in metals and ceramics[1–4]. Time-dependent cracking of quartz and granite has been experimentally demonstrated by Martin[5] and Wilkins[6] respectively, and slow growth of microfractures implicated in a number of geological phenomena such as pre-faulting dilatancy and magmatic intrusion[1].

However, we are not aware of any previous descriptions of chemical reactions accompanying cracking in naturally deformed rocks, or an identification of the reactions involved. The rocks to be described which exhibit intragranular fracturing accompanied by mineral reactions are located at the boundary of a major subvertical shear zone which transects granodiorite basement of the Aiguilles Rouge Massif, at Miéville, Switzerland. The granodiorite consists of orthoclase and plagioclase (~An_{25}–An_{18}) feldspar phenocrysts (45–60%), quartz (25–30%) and biotite (10–30%) as essential minerals. At increasing states of deformation the primary minerals are progressively reduced in grain size by a factor of 100–1,000 through microfracturing and recrystallisation. Accompanying the deformation is production of secondary albite, chlorite, epidote, calcite, ilmenite and sphene. The ambient temperature of cracking is estimated to be <250 °C from (1) the presence of prenhite–

pumpellyite facies cover rocks to the basement, which are involved in the shear zone[7], and (2) filling temperatures of 180–210 °C determined on primary fluid inclusions in feldspar fracture fillings. These represent minimum values inasmuch as corrections to temperature cannot be applied without precise knowledge of the hydrostatic pressure and fluid composition. However, corrections are probably less than +40 °C.

In rocks at a low state of deformation grains of biotite have intragranular fractures with a linear to sigmoidal shape which are bounded by an envelope of pale brown birefringence. This region corresponds to a decrease of Ti and increase of Fe relative to the composition of grains in areas unaffected by cracking. Fracture openings are occupied by secondary minerals, predominantly ilmenite, low-Ti biotite and high-Ti muscovite (Table 1). The presence of titanium in muscovite may be due to ultrafine grained disseminated ilmenite. These mineral products were also observed in kink bands, and at grain boundaries where the parent biotites have recrystallised, but are not present at grain boundaries in undeformed rocks. Parent grains and secondary products were examined using electron microprobe. Randomly orientated ilmenite grains (~10 μm long) are present up to 200 μm ahead of the leading edge of fractures (Fig. 1*a*). This region is characterised by the initiation of mineral reactions, and represents the first stage of chemically controlled disruption of the biotite structure where increase of the stress intensity at fracture tips may enhance lattice diffusion and thus catalyse reaction of the primary biotite. Decrease of volume accompanying the reaction of biotite to ilmenite plus muscovite in cracks may also serve to increase the local stress intensity at fracture tips, and enhance permeability.

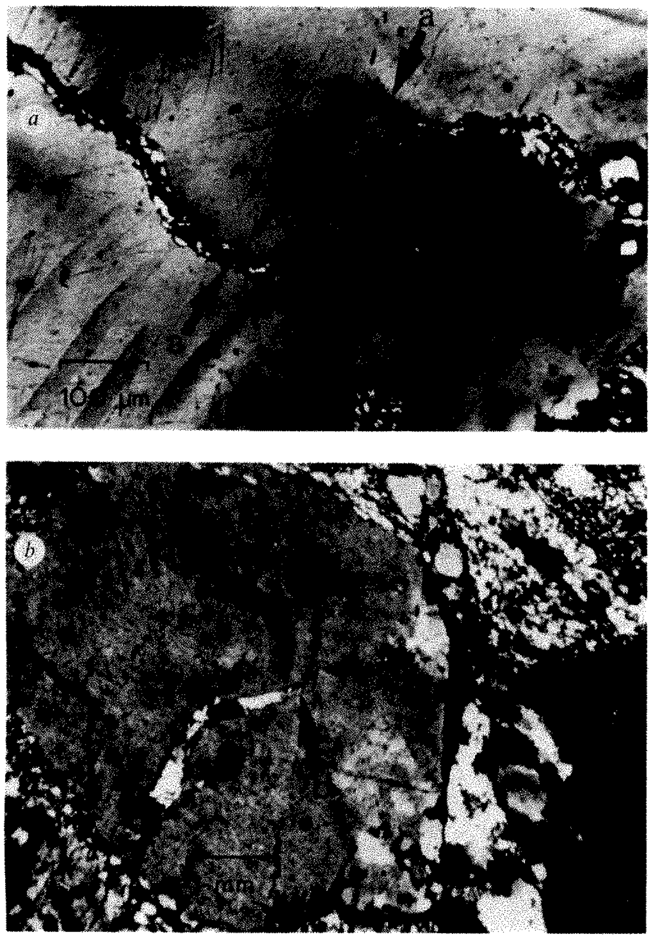

Fig. 1 *a*, Basal plate of biotite, within deformed granodiorite, containing fractures occupied by secondary ilmenite (a), muscovite and biotite. *b*, Phenocryst of orthoclase in a deformed granodiorite containing irregular non-displacive tensile fractures (b) occupied by secondary orthoclase, and/or albite; bounded on right hand margin by linear fracture with displacement.

Localisation of reactions ahead of fracture tips may be a diagnostic criterion for stress corrosion cracking, in contrast to alteration of minerals along fractures by means of the enhanced access of reactants. The reactions accompanying cracking described above do not involve any net hydration, as indicated by the relatively constant proportion of volatiles in rocks at all observed states of deformation, and from considerations of the structural water content of reactants and products in cracks. In addition, the δ^{18}O whole rock and ratio of $Fe^{2+}/\Sigma Fe$, both of which are sensitive to water-rock interaction, maintain constant values at increasing states of strain, implying no significant transport of fluids through the deforming rocks. However, the participation of an aqueous phase (as evidenced by the small quantities of fluids present in inclusions) is probably an important factor in catalysing reactions and the transport of components at fracture tips.

Grains of both orthoclase and plagioclase feldspar exhibit abundant microfractures of two distinct morphologies. First, linear fractures which terminate at grain boundaries, are narrow and involve transverse displacements of up to 3 mm (Fig. 1*b*). Second, irregular fractures which generally terminate within crystals, are relatively wide (20–200 μm), and have little discernible evidence of translation (Fig. 1*b*). The latter type of fractures are occupied by secondary reaction products of the parent grain, and are believed to have propagated by stress corrosion. Towards the boundaries and leading edge of irregular fractures parent grains become free of solid inclusions such as sericite, compared to regions remote from fracturing. During the cracking process sericite appears either to have migrated to the crack boundaries which it locally decorates, or to have reacted to secondary orthoclase in fractures, or to have been removed from the local chemical system. Secondary feldspar within fractures is clear, and contains abundant primary fluid inclusions in the sub-20 μm size range.

Dilatant fractures orientated normal to the local stretching direction are generally occupied by feldspar fibres growing in the direction of extension, bridging the fracture boundaries. In general, fractures are occupied with secondary feldspar of approximately the parent grain composition. However, many oligoclase phenocrysts recrystallise to a more sodic composition with corresponding loss of Ca, accompanied by loss of K as sericite is removed (Table 2). This reaction also proceeds during recrystallisation of oligoclase at grain boundaries[7]. Phenocrysts of orthoclase may have fracture fillings of orthoclase, plagioclase, or both feldspars coexisting, and this is also true for plagioclase phenocrysts (Table 2).

Stress corrosion cracking gives rise to grain size reduction in the rocks described and is probably operative in low to medium temperature environments where crystal plasticity is not generally predominant. However, its overall role in crustal deformation is not known. One possible consequence of this process is enhanced permeability, especially for reactions with a negative ΔV.

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Optical demonstration of crystalline superstructures in binary mixtures of latex globules

Sei Hachisu & Shigekuni Yoshimura

Institute of Applied Physics, University of Tsukuba,
Sakura Ibaraki 305, Japan

The colloid known as monodisperse latex[1] is an aqueous suspension of a synthetic polymer, the particles of which are ideally spherical and uniform in size (monodisperse). It shows phase transition[2] from a disordered to an ordered state, changing its appearance from milky white to iridescent. This is considered as experimental verification of the transition found by computer calculations[3,4] of concentrated hard core systems. Thus, monodisperse latex is a reliable model for studying the statistical mechanics of concentrated systems. Furthermore, as the particles are visible[5,6] by light microscopy when they are larger than 0.3 μm, we can now directly see what happens during phase transition and related phenomena. Recent work on the structure of gem opals[7] offers an important clue to understanding the statistical properties of binary systems. It showed that in some unusual opals two kinds of silica spheres of different size form a superstructure with long period. The structure must have been formed in a stable dispersion of silica spheres[7] and then been condensed and dehydrated. It should be possible to produce the structure artificially in some colloids and so provide an excellent model of binary systems. We report here that we have succeeded in producing superstructures in mixtures of two monodisperse polystyrene latexes of different particle size and have observed them microscopically[5] in their natural (stably dispersed) state. Several structures were found, including the alloy structures of $NaZn_{13}$ and $CaCu_5$ types, which belong to the so-called size-factor compounds[8].

The survey was made over a wide range of particle–number ratio for many combinations of latexes. For every mixture, the total solid content was adjusted to about 0.1 volume fraction, and their electrolyte (KCl) concentration was maintained at around 10^{-5} mol l^{-1}.

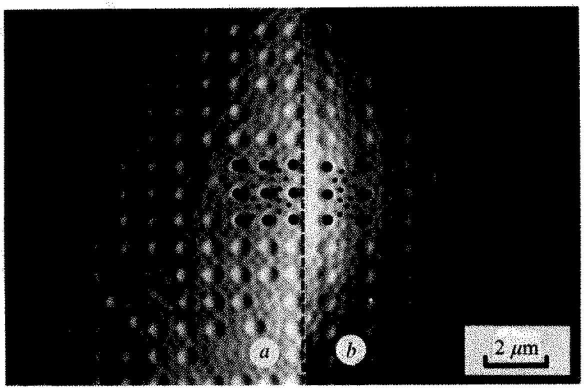

Fig. 1 Photomicrographs of the superstructure in a mixture of 5,500 Å and 2,700 Å latexes taken by light (metallurgical) microscope. Large spheres represent 5,500 Å particles and small ones 2,700 Å particles. Lattice type is simple cubic with respect to 5,500 Å particles. *a* Represents the (100) plane, and *b* the (110) plane. To distinguish the configuration of 2,700 Å particles, a superlattice is drawn. Staggered rhombuses can be seen in the (100) plane and a distorted pentagon in the (110) plane. The 2,700 Å particles were not static; from time to time they underwent a collective motion.

When conditions were optimal, in ten to several tens of hours after placing the cell on the microscope the ordered structures appeared. At this stage, the ordered regions were 'islands' about 50 μm in diameter surrounded by a 'sea' of irregular structure, but within a few days had grown to reach an equilibrium state in which the ordered structures coexisted with the disordered structure. These features were in accord with Sanders' observations on opal specimens[7].

The formation was quite reversible; the structures once formed could be broken by slight mechanical shock and reformed in several hours. The phenomenon resembled a kind of phase separation. The condition necessary for the ordered structures to appear is the basic problem of statistical mechanics of binary systems. It was rather strict, depending on the total solid content, the size ratio, the particle–number ratio and electrolyte concentration, and will not be discussed here. We will describe only two of the various structures discovered.

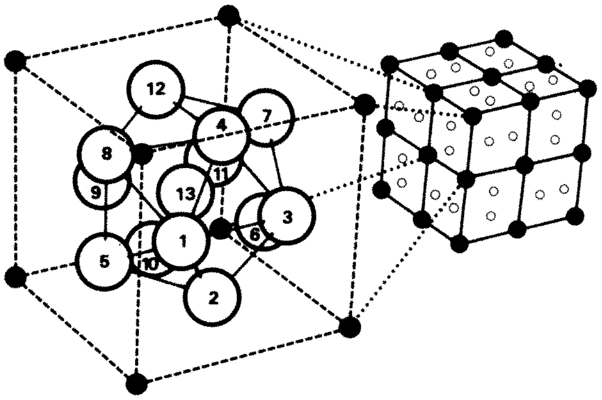

Fig. 2 Superstructure of the AB_{13}-type structure. Small solid circles indicate the position of the 5,500 Å particles and open circles represent the 2,700 Å particles forming a centred icosahedron. Particles 1, 2, 3 and 4 appear under the microscope as a rhombus in the (100) plane. Particles 2, 3, 4, 8 and 5 form a distorted pentagon in the (110) plane. The orientation of the icosahedron changes by 90° between adjacent cells.

Figure 1 shows patterns that appeared in the 5,500–2,700 Å system, the particle–number ratio of which was 1 : 8. These are, respectively, the (100) and (110) net planes of a simple cubic lattice with respect to 5,500 Å particles. The lattice constant was 0.85 μm. By moving the microscope focus upwards (an inverted-type microscope was used), we could find the next net plane. The mode of superposition of successive net planes and the measured spacing between them supported the simple cubic model.

The arrangement of the smaller particles is, however, rather complicated. The (100) plane contains four particles, which seem to be in the form of a rhombus (strictly speaking, it would be a shallow tetrahedron). The (110) plane contains six particles, five of which form a distorted pentagon with one particle at its centre. The centering particle must be situated at the body centre of the simple cubic lattice of 5,500 Å particles. Then, the simple cubic unit cell must contain four small particles in each of its side planes and one at its centre. This leads to the composition AB_{13} for this structure, in agreement with Sanders' opinion (personal communication).

The staggered arrangement of the rhombuses in the (100) plane indicates that the unit cell of the superstructure must be a block consisting of eight of the single cubic cells of 5,500 Å particles. The orientational behaviour of the distorted pentagon in the (110) plane is also interesting. These features are explained by assuming that the structure is as shown in Fig. 2. Note that this is the same, at least in particle configuration, as the structure of intermetallic compounds of $NaZn_{13}$ type[9].

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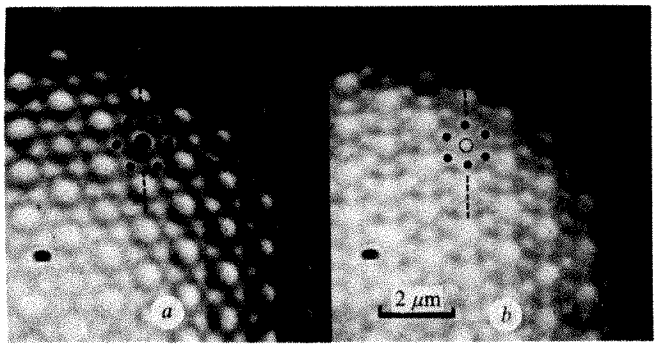

Fig. 3 Patterns observed in a 5,500–3,100 Å particle mixture. *a* Shows the pattern of the first plane, and *b* that of the second plane which lies about 4,000 Å above the first plane. Small particles in the first plane are at the centres of triangles of large particles, whereas those in the second plane lie at the midpoints of the lines connecting the blurred images of the larger particles. This is characteristic of an AB_5-type alloy structure.

Figure 3*a* shows a hexagonally ordered structure which appeared in a 5,500–3,100 Å system, the particle–number ratio of which was 1 : 2. On moving the microscope focus upwards by 0.8 µm, the next plane of the same arrangement appeared. All the particles, large and small, were directly above those in the first plane; no staggering was found. Then, moving the microscope focus down from this plane very carefully, another new plane was found lying between the above-mentioned two planes (see Fig. 3*b*), but in this new plane the smaller particles were arranged in a different way from those in Fig. 3*a*. The larger particles in Fig. 3*b* are out of focus, indicating that they are not in this plane. This new plane seems to consist solely of 3,100 Å particles, for if there were 5,500 Å particles here, they would have to lie between two infocused large particles whose centres are only 0.8 µm apart, which is impossible. Figure 4 shows the particle arrangement in the two planes. The superstructure must be composed of a combination of these two planes. Brief consideration leads to the formula AB_5 as the composition. This again corresponds to the structure of an intermetallic compound, in this case, $CaCu_5$ (ref. 10). This resemblance between the superstructure in latex and those of some alloys is very interesting. These latex superstructures may, therefore, be called 'colloid alloys'.

Because the electrolyte concentration is very low in the present systems ($\sim 10^{-5}$ mol l^{-1}) the colloid interaction between the particles must be repulsive, and in general, repulsive forces work to make the whole system uniform, and do not explain the occurrence of phase separation. Therefore, the mechanism of formation of these structures must be due to some entropy effect[2,3]. This means that in intermetallic compounds of this kind, the stoichiometry is determined by the size ratio of the component atoms; the electrons act only as a cement to hold the atoms together, and this cementing action can be replaced by pressure.

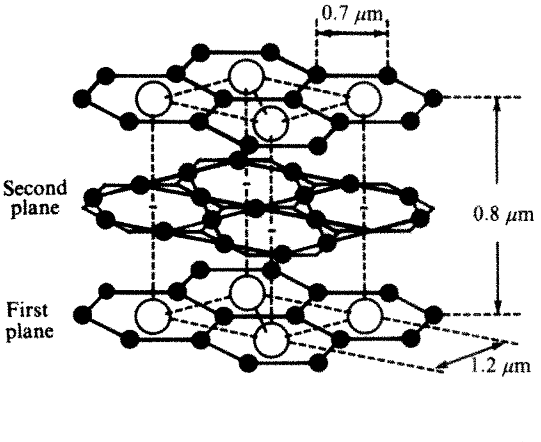

Fig. 4 Illustrative drawing of the AB_5-type structure. The interlayer spacing is exaggerated for convenience. Open and solid circles represent the 5,500 Å and 3,100 Å particles, respectively.

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Influence of stratospheric cooling from CO_2 on the ozone layer

I. S. A. Isaksen, E. Hesstvedt

Institute of Geophysics, University of Oslo, Norway

F. Stordal

Norwegian Meteorological Institute, Oslo, Norway

Perturbation of the atmospheric temperature distributio caused by an increased content of CO_2 is a key problem i climate research. The main concern is the increase in temperatures at the Earth's surface due to an increased greenhous effect. A general cooling of the stratosphere is predicted to tak place when CO_2 increases. A direct effect of lower temperature in the stratosphere is a change in the rates of some of th chemical reactions which control the ozone layer. Anthropogenic release of carbon dioxide may therefore lead to change in the ozone layer in the future. The present paper shows tha estimates of the temperature effect on ozone depend markedl on the level of chlorine in the stratosphere, as the catalytic ozon reduction by nitrogen and chlorine reactions respond differentl to changes in temperature.

Model estimates[1–3] predict an increase in the stratospheri ozone content with decreasing temperatures. This would mea that future decreases in stratospheric ozone due to fluorcarbo releases might have considerably fewer environmenta consequences due to an effect opposite to that of CO_2. I particular Groves *et al.*[3] find the effect from increased CO_2 to b significant compared with future chlorofluoromethane (CFM effects. To estimate the effect of reduced temperatures o stratospheric ozone, they used a one-dimensional time-dependent model with temperature feedback included. We have use a similar chemical transport model, with prescribed temperatur profiles, to repeat their calculations of the temperature effect o ozone. Our calculations give smaller changes in total ozone.

The one-dimensional time dependent model is the same a that used by Hessvedt and Isaksen[4], with updated rate constant Groves *et al.*[3] calculate the effect of increased CO_2 on tot ozone in a model which does not include chlorine reaction while our model takes the chlorine chemistry into account. T

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compare the models from a technical point of view, we calculated the effect of reduced temperatures corresponding to doubled CO_2 content in the atmosphere (600 p.p.m.), neglecting the chlorine chemistry. We also adjusted the rate constants for the more important reactions (the reactions of HO_2 with NO and O_3) in accordance with the values used by Groves *et al.*[3]. The resulting increase in total ozone density agrees well with the increase obtained by Groves *et al.*—5.6% compared with 5.5%. Calculated increase in ozone with height in the stratosphere is also found to be in good agreement in the two models. Thus the smaller increase in ozone obtained in our model is probably a result of a more complete ozone chemistry obtained through consideration of chlorine reactions. This point is discussed further below. Groves and Tuck[5] have recently shown that the temperature effect also becomes smaller when chlorine reactions are included in their model.

Future effects of CO_2 on ozone are obtained by two sets of calculations. Chlorine variations are the same in the two sets of calculations, determined by the release of CFMs, while we use two different temperature profiles. In the first set of calculations a standard temperature profile is used. In the second a different profile is used in the stratosphere, corresponding to doubling (600 p.p.m.) of CO_2 in the atmosphere. The difference in total ozone between the two sets of calculations at a certain year should therefore give the effect of the adopted temperature change for a chlorine content corresponding to that year. In which year this will take place, will depend on the future release rates of CO_2, Freon 11 and Freon 12 (F-11 and F-12). The temperature profile we adopt, corresponding to doubled CO_2 in the atmosphere, is the same as that calculated and used by Groves *et al.*[3]. It gives a temperature reduction of 4 °C at 25 km, 6 °C at 30 km, 10 °C at 40 km, and 13 °C at 50 km. These values refer to summer conditions. During the winter reductions are somewhat lower. The numbers are in good agreement with stratospheric cooling obtained by Manabe and Wetherald[6].

In the present model increase in the stratospheric chlorine content is calculated from a yearly release of F-11 and F-12 up to 1977 [7] which is approximately 85–90% of the production the same year. After 1977 constant release rates, equal to the 1977 production are adopted. Table 1 gives the calculated increase in total ozone density (in per cent) which would result from a doubling of the CO_2 in different years. As the change in ozone for each year is calculated with the same amount of chlorine present, the value in Table 1 gives the effect of temperature alone on ozone, at that certain level of chlorine.

The value for year 1955 refers to the chlorine content in the atmosphere before it was affected by release of F-11 and F-12. The source for chlorine before 1955 is assumed to be release of CH_3Cl and CCl_4. Both these compounds are assumed to be of natural origin and therefore to have constant release rates. For the years after 1955 Table 1 gives the amount of ClX, which is calculated to be present in the stratosphere above 30 km as a function of time. The temperature effect is seen to become less pronounced the more chlorine there is in the stratosphere. With the amount of chlorine estimated to be in the stratosphere in year 2040, a doubling of the CO_2 in the atmosphere leads to an increase in total ozone of 2.8%, slightly more than half the increase we obtain for a negligible chlorine content.

Table 1 Calculated increase in total ozone versus time resulting from the temperature changes given by a doubling of the CO_2 content in the atmosphere at various chlorine concentrations

Year		1955	2000	2020	2040
ClX (parts per 10^9)	0	1.0	2.6	3.5	4.4
$\Delta O_3/O_3$ (%)	5.1	4.3	3.3	3.0	2.8

The ClX ($Cl+ClO+HCl+ClNO_2$) mixing ratios are given for heights above 30 km, and are calculated in the model assuming a constant release rate of CFM at the 1977 level. The increases shown are for summer conditions.

Table 2 The ozone loss at various heights in the stratosphere in % of total loss at each height

	Chemical cycles			
Height (km)	Oxygen*	Hydrogen†	Nitrogen‡	Chlorine§
45	16.1	24.9	9.5	49.5
40	8.7	8.3	16.3	66.7
35	7.3	5.6	23.8	63.2
30	8.1	10.4	22.5	59.0
25	6.7	29.8	12.9	50.6
20	3.1	74.6	5.8	16.5

The loss in each group is obtained from the following reactions:
* Oxygen: $O+O_3 \rightarrow 2O_2$
† Hydrogen: $HO_2+O \rightarrow OH+O_2$ and $HO_2+O_3 \rightarrow OH+2O_2$
‡ Nitrogen: $NO_2+O \rightarrow NO+O_2$
§ Chlorine: $ClO+O \rightarrow Cl+O_2$
The values refer to the estimated stratosphere chlorine content for the year 2040, and are for the summer season.

We find this marked reduction of the effect of temperature changes, when the chlorine content is increased, to result from the different ways nitrogen and chlorine reactions respond to temperature changes. In the nitrogen cycle the ozone loss is proportional to the concentrations of NO_2. Reduced temperatures reduce the production of NO_2 from NO:

$$NO+O_3 \rightarrow NO_2+O_2 \qquad k_1=2.1\times10^{-12}\,e^{-1450/T} \qquad (1)$$

thereby lowering the equilibrium concentrations of NO_2. This makes the nitrogen cycle less effective, with the result of increased concentrations of ozone.

In the chlorine cycle where there is chemical equilibrium between Cl, ClO, and HCl, a change in the temperature profile first of all affects the reaction:

$$Cl+CH_4 \rightarrow HCl+CH_3 \qquad k_2=7.3\times10^{-12}\,e^{-1269/T} \qquad (2)$$

This reaction converts active chlorine, Cl and ClO, to HCl which is inactive with respect to ozone destruction. A temperature reduction in the stratosphere will therefore increase the ozone destruction by chlorine reactions. The calculations show that a temperature reduction of approximately 10 K at 40 km reduces the NO_2 densities, and thereby the ozone destruction through the nitrogen cycle by approximately 10%. It increases the ClO densities, and thereby the ozone destruction through the chlorine cycle by approximately 6%. Similar changes in NO_2 and ClO are found at other heights. Other species are found to be little affected by changes in temperature.

When the chlorine chemistry is considered in the model, the importance of the nitrogen cycle for the ozone distribution is markedly reduced. This will particularly be true when high chlorine build up takes place during the next century as we have predicted in Table 1. This is clearly illustrated in Table 2 where the loss (in relative units) due to the different chemical cycles is given. The numbers refer to the year 2040. In the region between 30 and 40 km where the temperature effect from a CO_2 increase has the largest impact on total ozone, the catalytic chlorine reactions are responsible for approximately 60% of the ozone destruction. It is therefore not surprising that the temperature effect on ozone is found to vary considerably with varying chlorine content in the stratosphere. A consequence of this is that comparisons of future effects of CO_2 and CFM also must take into account how long it takes for CO_2 to double its atmospheric concentrations, and how accurate the adopted release rates of CFM are. In these comparisons we have assumed that CO_2 will reach 600 p.p.m. around year 2040. Unfortunately this assumption, and also the assumption of a future release rate of CFM on the 1977 level are both highly uncertain.

In order to compare the effect of future increase in atmospheric CO_2 with the effect of increased CFM levels, estimated reductions of total ozone due to constant future CFM release on the 1977 level are given in Table 3. The calculations show that there will be a marked decrease in ozone well into the next

Table 3 Calculated changes in total ozone concentrations versus time due to the release of CFMs (F-11 and F-12)

Year	1978	2000	2020	2040	$\rightarrow\infty$
$\Delta O_3/O_3$ (%)	−1.9	−7.4	−11.1	−13.9	−23.4

Release rates up to 1977 are taken from ref. 7. A constant release rate correponding to the production in 1977 is adopted for the years after. Effects from changes in stratospheric temperatures are neglected. The changes shown are for the summer season.

century. The reduction due to CFM release in year 2040, which is of special interest when comparisons with the CO_2 effect is made, is approximately 14%. Compared with the 2.8% increase in total ozone from a doubling of CO_2 we obtained above, we find the temperature effect due to increased CO_2 to be only an adjustment of the CFM effect. Note, however, that the above values are likely to represent overestimates of the CFM effect as we have not considered any feedback from reduced O_3 in the stratosphere to the temperature profile. However, estimates of this effect[8] indicate that it leads to a small change in the CFM reduction of ozone. The calculations of Groves and Tuck[5] show considerably smaller future reductions in total ozone when the combined effect of CO_2 and CFM is considered, than those obtained in our calculations.

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Nitrogen fertiliser and stratospheric ozone: latitudinal effects

R. C. Whitten*, W. J. Borucki*, L. A. Capone†, C. A. Riegel† & R. P. Turco‡

* Ames Research Center, NASA, Moffett Field, California 94035
† San Jose State University, San Jose, California 95192
‡ R & D Associates, Marina del Rey, California 90291

Substantial increases in atmospheric nitrous oxide (N_2O) resulting from increased use of nitrogen fertilisers might cause large (to 10%; ref. 1) and unacceptable decreases in the stratospheric ozone content[1–3]. Such ozone decreases would be caused by catalytic reaction cycles involving odd-nitrogen ($NO_x \equiv NO + NO_2$) which is formed by N_2O decomposition in the upper stratosphere[1]. However, Turco *et al.*[4], using a background chlorine level of 2 p.p.b.v. (parts per billion by volume), show that if the recently measured values of the reactions in Table 1 are used, a 50% increase in N_2O would lead to a 2.7% increase in the stratospheric column density, although the ozone content above 30 km would be reduced by > 5%; they also estimated (unpublished data) that the change in the ozone column density caused by doubling the N_2O abundance would be very close to zero (within about 0.1%). Here we extend these calculations of N_2O/ozone effects to two dimensions, thereby delineating the latitudinal dependence expected for such ozone perturbations; we also discuss the effects of changes in stratospheric chlorine levels ($Cl_x \equiv Cl + ClO + HCl$) on predicted ozone changes.

The two-dimensional model used in this study extends from the surface to 60 km altitude and from latitude 80° N to 80° S with 2.5 km vertical grid and 5° latitudinal grid. About 35 constituents in the oxygen–nitrogen–hydrogen–chlorine–carbon families are included. Transport is simulated by advection and eddy diffusion. Detailed discussions of the model are given elsewhere[7,8].

We have made model calculations that simulate a doubling of atmospheric N_2O for two possible levels of stratospheric chlorine content, 1.4 p.p.b.v. and 2.1 p.p.b.v., at 60 km altitude. The corresponding predicted changes in ozone abundances are shown in Fig. 1 as a function of altitude and latitude. The most notable feature of these profiles is the substantial ozone increase predicted for the lower stratosphere (up to 10% below 20 km at high latitudes, and up to 15% below 27 km at low latitudes). The ozone gains are caused principally by the interference by enhanced NO_x concentration in the HO_x reaction cycle that consumes ozone; that is, through interference caused by the coupling reaction (see Table 1)

$$NO + HO_2 \rightarrow NO_2 + OH \qquad (1)$$

This occurs via two major mechanisms. One is accelerated HO_x recombination through reactions involving nitric acid

$$(OH + NO_2 \xrightarrow{M} HNO_3;\ OH + HNO_3 \rightarrow H_2O + NO_3).$$

The second suppresses HO_x catalysis of ozone by direct short circuiting of the reaction

$$HO_2 + O_3 \rightarrow OH + 2O_2 \qquad (2)$$

by reaction (1) (see Table 1). Eventually, as NO_x is increased beyond some level, enough of the OH is taken up by nitric acid and destroyed by the reaction $OH + HNO_3$ that it decreases as NO_x is increased. Our computations, in fact, show that OH may be reduced by nearly 10^6 molecules cm^{-3} (by about 25%) for N_2O doubling, especially at mid-latitudes and high latitudes. Reductions in OH have, of course, consequences for the lifetimes of other stratospheric constituents, including many hydrocarbons and halocarbons.

Figure 2 shows the predicted latitudinal variation of the relative (per cent) ozone column density change for two levels of stratospheric chlorine (1.4 p.p.b.v. and 2.1 p.p.b.v.). The important features to be noted here are the minima that may occur at low latitudes, and the pronounced influence of the ambient stratospheric chlorine level. The low latitude minima would occur partly because of stratospheric transport, which

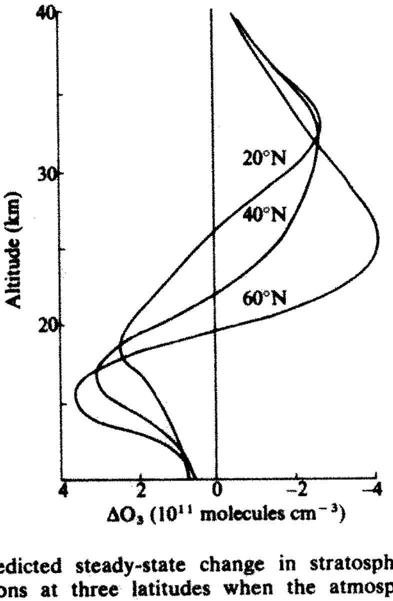

Fig. 1 Predicted steady-state change in stratospheric ozone concentrations at three latitudes when the atmospheric N_2O abundance is doubled. The level of stratospheric chlorine is 2.1 p.p.b.v.

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Table 1 Some key reactions

Reaction	Rate coefficient*	Ref.
(1) $NO+HO_2 \rightarrow NO_2+OH$	8×10^{-12}	5
(2) $HO_2+O_3 \rightarrow OH+2O_2$	$1.4\times10^{-14}\exp\left(\frac{580}{-T}\right)$	6

T is the absolute temperature in degrees Kelvin.

moves relatively inert substances (tracers), such as ozone and total odd-nitrogen, from low to high latitudes[9]; as a result, NO_x increases at higher latitudes. On the other hand, increases in the background level of chlorine are predicted to cause less ozone destruction due to increase of N_2O, mainly because of the reaction

$$NO+ClO \rightarrow NO_2+Cl \tag{3}$$

and to a lesser extent through the formation of chlorine nitrate ($ClONO_2$). As NO_x is increased, this strong Cl_x–NO_x coupling reaction significantly slows the rate of ozone catalysis by ambient chlorine. In other words, the addition of NO_x can render the ambient chlorine catalytic cycle less effective through reaction (3) and can, if the chlorine concentration is high enough, result in an ozone increase. Hence the complex interplay among the NO_x, HO_x, and Cl_x reaction cycles (also discussed in ref. 10) at first leads to a net ozone increase as N_2O burdens are increased, which later 'saturates' and becomes a net ozone decrease. This effect was predicted by Turco *et al.*[4] for aircraft injections of NO_x into the stratosphere. The present calculations show that the saturation point is indeed very sensitive to the ambient chlorine level, and is not uniform over the globe. Note also that this prediction is highly sensitive to other model parameters, including the ambient water vapour concentration and the rates of several key reaction processes such as reactions (1) and (2). Qualitative estimates of the rates of odd-oxygen (ozone) consumption for the key catalytic reaction steps are presented in Fig. 1 of ref. 4. The global average ozone changes shown in Fig. 2 are roughly consistent with those obtained with our one-dimensional model[4]. Note that the hemispherical asymmetry in Fig. 2 is a consequence of the asymmetry in eddy transport required for matching observed ozone abundances by model predictions. The small differences between the two models are due mainly to differences in effective vertical transport. That is, for the high chlorine case, we obtain an annually and globally averaged ozone change of about −0.5%; the corresponding prediction with the one-dimensional model is zero change. The difference is due to a somewhat different mean vertical transport rate between the two models.

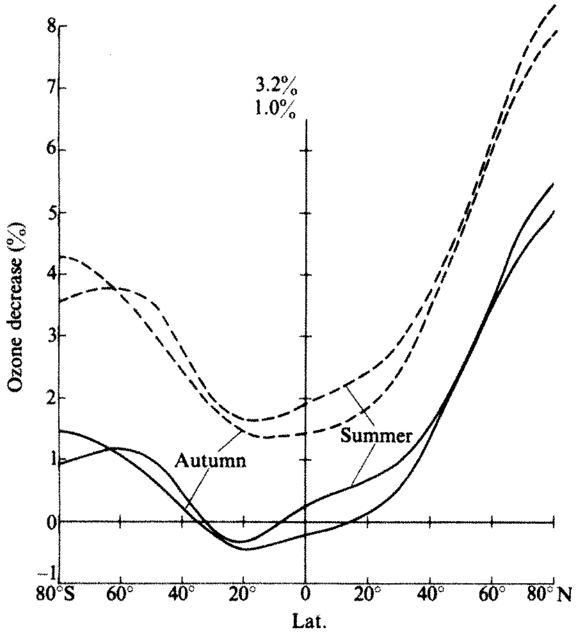

Fig. 2 Predicted latitude dependence of the change in the ozone column density for the summer and autumn seasons and for two levels of stratospheric chlorine: $Cl_x = 1.4$ (dashed line) and 2.1 p.p.b.v. (solid line).

The cycle of atmospheric nitrous oxide is not well understood. A major controversy centres on the possibility of a large unidentified tropospheric sink for N_2O. If the magnitude of that sink is as large as has been suggested by Hahn[11], it is unlikely that the abundance of atmospheric N_2O will double in the next 50 years. Inasmuch as stratospheric levels of chlorine are expected to exceed about 2 p.p.b.v. in future decades, our calculations suggest that global ozone reductions due to large N_2O increases could be quite small, and that they should be most pronounced at high latitudes; at low latitudes, ozone column increases are actually likely to occur. Thus, because the atmospheric lifeline of N_2O is probably not much larger than 10 years, we apparently have the time to carry out an extended and detailed scientific analysis of the global N_2O budget before critical decisions concerning the regulation of nitrogen fertilisers will be necessary.

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Ethylene evolution by thrips-infested cowpea provides a basis for thrips resistance screening with ethephon sprays

H. C. Wien* **& C. Roesingh†**

International Institute of Tropical Agriculture, PMB 5320,
Ibadan, Nigeria

Pests are major constraints to increased cowpea production in tropical subsistence agriculture where cash inputs are small. Breeding for pest resistance is therefore the major component of pest management strategies for crop improvement. Of paramount economic importance among these pests are flower thrips [*Megalurothrips sjostedti* (Tryb.)] which may cause abscission of flower buds, flowers and peduncles, leading to seed yield losses of up to 100% (refs 1, 2). It has been difficult to use available sources of moderate thrips resistance in a breeding programme because of thrips variation in time and space in field screening[3]. This paper reports that ethylene is produced when thrips infest cowpea peduncles. We have used this fact to develop a screening technique using the synthetic growth regulator ethephon (2-chloroethylphosphonic acid), which when sprayed on the plant is translocated to actively-growing tissue, where it breaks down to form ethylene[4–6]. Cultivars susceptible to abscission caused by thrips also show increased abscission with ethephon treatment. The technique may be useful in identifying sources of resistance to other abscission-causing agents.

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* Present address: Department of Vegetable Crops, Cornell University, Ithaca, New York 14853.
† Present address: Institut für Phytomedizin, Universität Hobenheim, Stuttgart, FRG.

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Table 1 Peduncle abscission and flower budset on the main stem of lines with contrasting resistance level to *Megalurothrips*, when sprayed with one of three concentrations of ethephon in two field experiments

	Peduncle abscission (%)						Flower budset (%)					
	Expt 1			Expt 2			Expt 1			Expt 2		
Ethephon (p.p.m.)	0	200	400	0	200	400	0	200	400	0	200	400
Five thrips-susceptible cultivars	1.2	48.6	67.0	14.4	78.8	91.4	54.6	25.4	12.8	49.8	5.0	1.6
Five thrips-resistant cultivars	0.4	7.4	22.6	0	7.0	35.6	71.0	62.6	30.4	76.2	50.4	15.6
Mean of 30 cultivars	1.2	35.7	53.3	4.0	50.2	75.0	62.9	32.9	16.5	62.9	19.4	5.7
s.e.m., sprays		6.2			1.9			6.0			2.1	
s.e.m., cultivars		6.0			4.8			5.7			4.3	
c.v. (%)		49.1			27.0			37.5			36.4	

Evidence that thrips damage can lead to ethylene production was obtained when pot-grown plants of two cowpea cultivars (*Vigna unguiculata* L. Walp.), raised in insect-free conditions, were transferred to a thrips-infested cowpea field and ethylene evolution of the peduncles was measured. Half the plants were treated with the systemic insecticide monocrotophos 1 day before transfer to the field. The insecticide-treated plants remained free of thrips, while unsprayed plants showed an average of three adult thrips and six nymphs per peduncle 4 days after field transfer. At that time, when flower bud abscission of the unsprayed plants was just beginning, three peduncles were removed from six plants in each treatment and incubated at 23 °C in 10-ml plastic syringes under 0.75 bar suction tension. To absorb CO_2 given off in respiration, 0.2 g solid KOH was placed in a perforated plastic vial in each syringe. After 24 h incubation, a 0.5-ml gas sample from the syringe was injected in a gas chromatograph for determination of the presence of ethylene[7]. Thrips-infested peduncles produced 640 nmol ethylene per g fresh weight per 24 h and the controls produced 152 nmol. The differences are significant at the 1% level. There was no difference between the two cultivars.

Therefore, it seems that thrips infestation leads to ethylene evolution. As no attempt was made to remove the thrips from infested peduncles before incubation, the source of the ethylene still remains to be determined. The most likely source would be ethylene evolved by the outer cell layers of the flower buds and peduncles that had been injured by the thrips[8,9]. Ethylene could also be produced by the plants as a result of interactions with chemicals secreted by the insects, as occurs in cotton[10].

Can applications of the ethylene-producing chemical ethephon simulate thrips damage, and identify cultivars resistant to thrips? To answer these questions two field experiments, comparing flower bud and peduncle abscission of 30 cowpea lines were planted in first and second rains at IITA, Ibadan. Plots consisted of single rows 1 m apart and 3 m long, replicated four times. Both experiments were sprayed weekly with insecticides starting at 2 weeks after sowing. Applications of 0, 200 or 400 p.p.m. ethephon in water containing 0.5% (v/v) of a wetting agent formed the main plots, with cultivars as subplots in the split plot experiments. In experiment 1, a single ethephon spray was applied at about 1 week before anthesis of a particular cultivar. At this stage, the oldest peduncles were about 1 cm long. In experiment 2, all cultivars were sprayed 34, 39 and 47 days after sowing with 0, 200 or 400 p.p.m. ethephon, to ensure that each cultivar received at least one spray during the period of maximum sensitivity to abscission. In both experiments the five lowest peduncles on the main stem of three plants per plot were examined for peduncle or flower bud abscission 2–4 days after the controls started flowering. Only the lowest two buds per peduncle were considered in rating flower bud abscission. The number of plants flowering was counted on each plot at 2–3 day intervals.

The same 30 cultivars were field tested for thrips resistance in four replications in late second rains without insecticide protection (experiment 3). Flower thrips infestation was heavy and completely prevented flowering of some cultivars. After 50 days, thrips susceptibility was measured by counting the number of abscised peduncles on the first five nodes of the main stem of three plants per plot.

In experiments 1 and 2 ethephon sprays resulted in increased peduncle abscission and reduced budset in all cultivars, with thrips-susceptible cultivars being most severely affected (Table 1). Thrips-resistant cultivars showed less damage than susceptible lines with 200 and 400 p.p.m. ethephon (spray treatment × cultivar interaction was significant at the 1% level). There was no evidence of enhanced fruitset at concentrations of 250–500 p.p.m. as reported for *Phaseolus vulgaris*[11].

The correlation between per cent peduncle abscission due to ethephon and that due to thrips was 0.75 and 0.62 for experiments 1 and 2, respectively (Fig. 1). In both experiments, five cultivars showed good levels of resistance to ethephon and to thrips, indicating that selection of plants with low main stem peduncle abscission after sprays of 400 p.p.m. ethephon can identify individuals with thrips resistance. Five other cultivars showed low incidence of peduncle abscission due to thrips after one spray of 400 p.p.m. ethephon, but less tolerance to repeated applications of the chemical. Such cultivars may be less resistant to higher thrips populations than occurred in experiment 3.

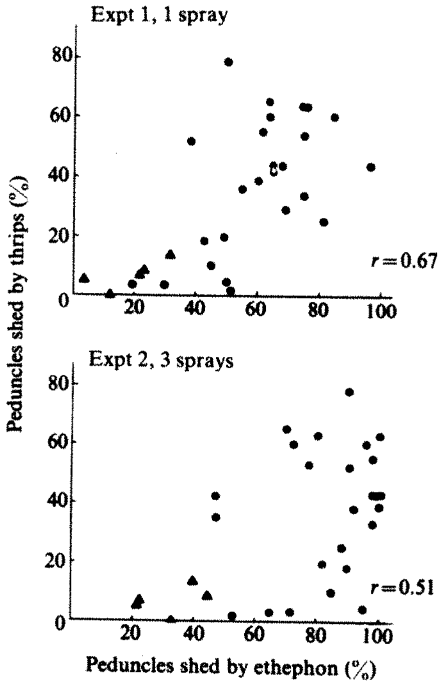

Fig. 1 The relationship of peduncle abscission caused by thrips and by 400 p.p.m. ethephon for 30 cowpea lines grown in two field experiments. Points marked by a triangle represent lines that have shown consistent thrips resistance in other experiments.

It seems clear that ethephon sprays can assist in identifying cowpea lines which possess flower thrips resistance. The sprays can be applied uniformly, and as resistance is built up through intercrosses of different resistant parents, the dosage level could be increased to simulate heavier thrips pressure. The thrips resistance of such material would require verification, for in large plantings of a resistant cultivar, thrips levels may build up sufficiently to cause damage.

The use of ethephon as a screening tool for abscission resistance could have important implications for other crop improvement programmes. Various insect pests are known to cause abscission of young reproductive structures in cotton[11-13] and plum[14], while drought, high temperatures and shade may cause similar damage in edible legumes[7,15,16]. Increases in evolution of ethylene may result from insect damage[11], shading, drought[17,18] or plant disease[19].

Thus the abscission response of cultivars to different levels of applied ethephon might identify lines with better tolerance to such pests and stress conditions. Although some authors have pointed out cultivar differences with regard to abscission of reproductive structures[20,21] the systematic screening for cultivars less susceptible to abscission has to our knowledge not been previously reported and seems a promising way of overcoming the problems of abscission-causing insect pests and physical factors.

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Is prostacyclin a physiologically important circulating anti-platelet agent?

Michael L. Steer*, D. Euan MacIntyre*†, L. Levine‡ & Edwin W. Salzman*

* Department of Surgery, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215
‡ Department of Biochemistry, Brandeis University, Waltham, Massachusetts 02154

Prostacyclin (PGI_2) is a naturally occurring arachidonic acid metabolite which causes relaxation of vascular smooth muscle[1] and is the most potent inhibitor of platelet aggregation known[2]. The anti-aggregatory effects of PGI_2 result from its ability to stimulate platelet adenylate cyclase and elevate platelet cyclic AMP levels[3,4]. The possibility that PGI_2 is a circulating hormone that acts to inhibit platelet aggregation *in vivo* was first suggested by Gryglewski *et al.*[5] and Moncada *et al.*[6]. In their studies using heparinised rabbits and cats, exogenous PGI_2 caused a decrease in superfused *ex vivo* tendon weight, and this effect was inhibited by infusion of antiserum directed against a stable PGI_2 analogue (5,6-dihydro-PGI_2). Tendon weight gain occurred in the absence of exogenous PGI_2 and was enhanced by infusion of PGI_2-binding antibodies, an effect that was more pronounced in arterial than in venous blood. In contrast, Smith and coworkers[7] observed that their PGI_2-binding antibodies (antiserum directed against 6-keto-$PGF_{1\alpha}$) suppressed the vasodepressor effects of exogenous PGI_2 infused into cats but did not alter blood pressure in the absence of exogenous PGI_2. They concluded that PGI_2 is not a circulating vasodepressor hormone. The present studies were designed to examine the physiological role, if any, of circulating PGI_2 in the regulation of human platelet function. Here, we report the results of experiments dealing with the effects of PGI_2 and antibodies directed against 5,6-dihydro-PGI_2 on human platelet function and cyclic AMP levels. These studies indicate that, in humans, PGI_2 is not a physiologically important circulating inhibitor of platelet aggregation.

Antibodies which neutralise the effects of PGI_2 were raised in rabbits using the stable PGI_2 analogue $6\alpha,H$-5,6-dihydro-PGI_2 (PGI_1) as the hapten. The preparation of these antibodies and their detailed serological specificity has been reported previously[8]. The γ-globulin fractions of the immune plasma (anti-PGI_1) and control rabbit plasmas were purified by precipitation with 40% ammonium sulphate. The insoluble proteins were dissolved in 0.14 M NaCl and 0.01 M Tris (*p*H 7.4) containing 0.1% gelatin and dialysed against this buffer. The final γ-globulin concentration was equivalent to that in the unextracted plasma. Platelet aggregation was quantified by measuring the maximal slope of aggregation after addition of ADP[9]. Platelet cyclic AMP was measured using a commercially available radioimmunoassay kit after acetylation of the unlabelled cyclic AMP[10]. In the cyclic AMP experiments, platelets were separated from plasma by centrifugation through a layer of silicone oil into trichloroacetic acid (TCA). This method, which results in >90% platelet recovery in the TCA layer and separates platelets from plasma in less than 1 min, will be described in detail elsewhere. (M.L.S., in preparation).

Addition of PGI_2 (1 nM) to platelet-rich plasma (PRP) more than doubled the platelet level of cyclic AMP at the first time measured (2.5 min) (Fig. 1). Platelet aggregation induced by

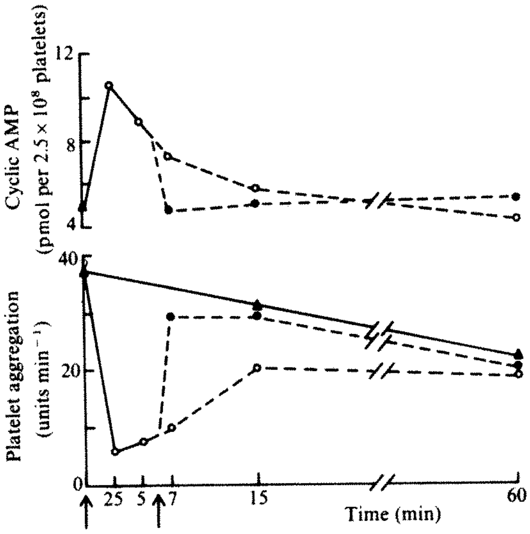

Fig. 1 Typical experiment showing the effect of PGI_2 and antibodies that bind PGI_2 on platelet cyclic AMP levels and ADP-induced platelet aggregation. PGI_2 (1 nM) was added to the PRP at zero time (1st arrow) and 50 μl ml^{-1} of either γ-globulin from an immune rabbit (anti-PGI_1) or γ-globulin from normal plasma was added at 6 min (2nd arrow). Cyclic AMP and platelet aggregation were measured as described in the text. Values represent the mean of duplicate determinations which differed by less than ±10%. ●, Anti-PGI_1; ○, normal plasma; ▲, no addition.

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† To whom correspondence should be addressed.

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Table 1 Effect of anti-PGI_2 on the increase in rate of ADP-induced aggregation after rapid preparation of platelet rich plasma

Time after collection (min)	Slope of aggregation (units min^{-1})		Ratio of aggregation slopes at 5 or 10 min versus 3 min	
	Control	Antibody	Control	Antibody
Arterial				
3	20.1±11.0	18.5±11.4	—	—
5	24.7±8.3†	23.6±10.8*	1.39±0.37	1.42±0.44
10	27.6±11.1†	25.4±10.4†	1.51±0.36	1.59±0.55
Venous				
3	23.4±10.8	26.1±9.1	—	—
5	28.4±11.2†	31.6±11.3*	1.25±0.20	1.22±0.19
10	32.9±9.0†	32.6±10.4†	1.57±0.66	1.29±0.16

The PRP was separated from whole blood by high-speed centrifugation. PRP preparation was completed within 2.5 min of blood collection, and 50 µl ml^{-1} PRP of either immune or control γ-globulin was added immediately thereafter. This amount of immune γ-globulin had previously been found to reverse the anti-aggregatory effects of 3 nM exogenous PGI_2. Aggregation, induced by 2 µM ADP, was measured 30 s, 2.5 min and 7.5 min later (that is, 3, 5, and 10 min after blood collection). Results represent mean ±s.d. of duplicate measurements from either 7 (arterial) or 11 (venous) separate experiments using different donors, none of whom had ingested aspririn-containing drugs during the 2-week period before testing. None of the blood samples contained heparin. The slopes of aggregation at 5 min and 10 min with or without antibodies were compared with the comparable values at 3 min. The statistical significance of differences was evaluated using the *t*-test for paired data. Symbols indicate $^*P<0.05$ or $^\dagger P<0.01$ in this comparison. The 5- and 10-min slopes of aggregation were significantly greater than that at 3 min, but there was no difference between controls and samples containing antibodies in this regard.

ADP (2 µM) was almost totally inhibited at this time. Over the next hour, platelet cyclic AMP levels gradually fell to the original level, while the rate of ADP-induced aggregation rose to the control level. Addition of anti-PGI_1 antibodies 6 min after PGI_2 accelerated the fall in cyclic AMP level and the rise in the rate of ADP-induced aggregation, both of which approximated the initial values within 1 min of addition of the antibody. In contrast, addition of the γ-globulin fraction of plasma from a non-immune rabbit had no effect. Addition to control PRP of immune γ-globulin alone, or of PGI_2 previously incubated with these antibodies, did not alter platelet cyclic AMP levels or ADP-induced aggregation. Thus, the anti-PGI_1 antibodies rapidly reverse the anti-aggregatory effects of exogenous PGI_2.

To investigate whether the sensitivity of platelets to PGI_2 differed in PRP and in whole blood, concentration–response curves for inhibition of ADP-induced aggregation were obtained by addition of exogenous PGI_2 to PRP directly and to whole blood before rapid preparation of PRP. The curves in PRP and in whole blood were virtually identical, and the threshold concentration for inhibition of aggregation was 0.1 nM PGI_2 in both cases. Addition of anti-PGI_2 antibodies to whole blood 15 s after the addition of exogenous PGI_2 (0.1–1 nM) negated the inhibitory effects on platelet aggregation.

If PGI_2 is a physiologically important circulating inhibitor of platelet aggregation, reduction in PGI_2 levels should enhance aggregation. PGI_2 is normally degraded in the circulation with a half-time of 3–5 min (ref. 11), although in citrated plasma the half-time is prolonged to 25–30 min (D.E.M. and E.W.S., unpublished). Previous reports have demonstrated that platelet reactivity *in vitro* increases with time after removal of blood from the vascular system[12,13]. This phenomenon might be explained by time-dependent degradation of PGI_2. We have also found that the rate of ADP-induced aggregation increases with time after rapid preparation of PRP followed by incubation at 37 °C (Table 1). However, immediate addition of anti-PGI_1 antibodies to PRP prepared within 3 min of venous or arterial puncture did not enhance the rate of ADP-induced aggregation at that time or alter the increase in aggregation over the next 10 min. The PGI_2-binding antibodies failed to enhance aggregation in either arterial or venous blood (Table 1).

The present studies demonstrate that: (1) the plasma or blood concentration of PGI_2 required for inhibition of ADP-induced aggregation is ≥0.1 nM; (2) inactivation of PGI_2 by addition of PGI_2-binding antibodies to PRP or to whole blood rapidly (<1 min) reverses the effects of exogenous PGI_2 on platelet aggregation and platelet cyclic AMP; (3) addition of PGI_2-binding antibodies to PRP shortly (<3 min) after either arterial or venous blood collection does not alter platelet aggregation.

If PGI_2 were a physiologically important circulating inhibitor of platelet aggregation in humans, addition of antibodies that neutralise PGI_2 should have enhanced aggregation in these conditions. We conclude that the circulating concentration of PGI_2 in humans is below that required for inhibition of aggregation (<0.1 nM) and that PGI_2 is not a circulating anti-platelet agent in normal conditions in humans. The time-dependent increase in platelet responsiveness after blood collection is therefore not due to degradation of circulating PGI_2.

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Prostacyclin inhibits mobilisation of fibrinogen-binding sites on human ADP- and thrombin-treated platelets

Jack Hawiger, Spencer Parkinson & Sheila Timmons

Departments of Pathology and Medicine, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 and Boston VA Medical Center, Boston, Massachusetts 02130

Prostacyclin (prostaglandin I_2, PGI_2), produced by the blood vessel wall is the most potent known inhibitor of platelet aggregation induced by such stimuli as ADP and thrombin[1]. It binds to a specific platelet receptor[2] and activates adenylate cyclase, raising the cyclic AMP level in platelets[3,4]. This property can be important because platelets participate in several significant interactions. For example, the interaction with fibrinogen or fibrin contributes to the formation of the haemostatic plug[5]. Intact plasma fibrinogen is required for the aggregation of platelets induced by ADP[6], and endogenous platelet fibrinogen influences thrombin-induced aggregation[7]. We have therefore studied the effect of prostacyclin on the interaction of fibrinogen with human platelets. We now report that prostacyclin inhibits the mobilisation of specific binding sites ('receptors') for fibrinogen on human platelets and that this effect parallels the inhibition of ADP- or thrombin-induced aggregation. The inhibitory effect of prostacyclin may limit the extent of platelet–fibrinogen interaction *in vivo* and in extracorporeal circulation.

We isolated platelets from human blood and separated them from plasma proteins by stepwise albumin gradient centrifugation and Sepharose 2B gel filtration as before[8]. All experiments were performed with platelets suspended in HEPES buffer, *p*H 7.35, containing 0.1% dextrose and 0.35% albumin. Aggregation was measured photometrically in a Payton (Buffalo, New York) dual channel aggregometer according to Born's method[9]. Human plasma fibrinogen (Kabi) was prepared and labelled with ^{125}I as before[10]. This preparation was 95% clottable with thrombin. Solutions of PGI$_2$, 6-keto PGF$_{1\alpha}$ and PGD$_2$ were prepared in cold Tris-HCl buffer, *p*H 9.37, immediately before use.

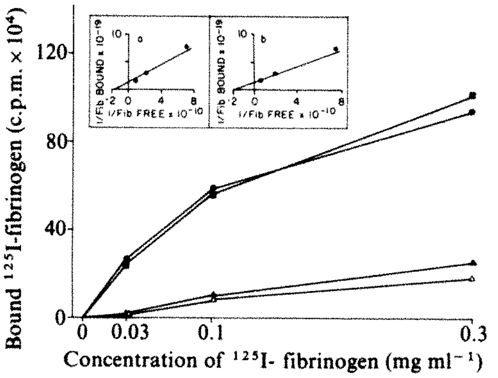

Fig. 1 Binding of ^{125}I-labelled human fibrinogen to platelets exposed to: ■, 4.5 μM ADP; ●, thrombin (0.05 U ml^{-1}) followed by hirudin (0.1 U ml^{-1}); ▲, buffer; △, thrombin or ADP and a 20-fold excess of unlabelled fibrinogen. Each incubation included 1 × 10^8 platelets and the specified amounts of fibrinogen (4.3 × 10^4 c.p.m. μg^{-1}) in 0.5 ml of HEPES buffer, *p*H 7.35, containing 0.1% dextrose and 0.35% albumin. The insets contain double reciprocal plots of binding data for platelets stimulated by ADP and thrombin, respectively. Specific binding was calculated after subtracting values of 'nonspecific' binding in the presence of an excess of unlabelled fibrinogen.

Binding of fibrinogen to platelets was achieved in 0.5-ml reaction mixtures containing 1 × 10^8 platelets with 50 μl of ADP or thrombin preceded or followed by 50 μl of ^{125}I-fibrinogen. To examine the effect of prostaglandins, platelet suspensions were pre-incubated for 3 min with 50 μl of prostaglandin or buffer before the addition of the other agents. Incubation was at room temperature (22 °C) in polypropylene tubes (12 × 75) without stirring. After 15 min the mixture was centrifuged through a 9 : 1 (v/v) mixture of dibutylphthalate and Apiezon oil C (ref. 11). The supernatant was sampled for free radioactivity, and bound radioactivity was measured in the platelet pellet cut off together with the tip of the tube after rapid freezing of its contents in a mixture of dry ice and acetone.

Before examining the effect of prostacyclin on the interaction of ^{125}I-fibrinogen with human platelets, it was necessary to establish the basic characteristics of binding of ^{125}I-fibrinogen to human platelets. Intact, resting platelets bound very little ^{125}I-fibrinogen (Fig. 1). After stimulation with 4.5 μM ADP, there was a four- to fivefold increase in the amount of ^{125}I-fibrinogen bound. Binding to platelets stimulated with ADP approached saturation at 300 μg of ^{125}I-fibrinogen, and equilibrium binding was achieved within 5 min. Approximately 70% of the labelled fibrinogen could be displaced by a 20-fold excess of unlabelled fibrinogen, suggesting that binding was specific. Moreover, adenosine, AMP and ATP in equimolar concentrations did not increase the binding of ^{125}I-fibrinogen to platelets (not shown). The amount of ^{125}I-fibrinogen bound was calculated from a double reciprocal plot[12] of the binding data obtained after subtracting values for nonspecific binding (Fig. 1, left-hand inset). If we assume uniform labelling of fibrinogen and a molecular weight of 340,000 (ref. 13), we can estimate that 50,000 molecules of ^{125}I-fibrinogen per platelet were bound to ADP-stimulated platelets with the apparent dissociation constant of 1.5×10^{-7} M.

Thrombin in trace concentrations also stimulates human platelets[14]. To gain further insight into the mobilisation of fibrinogen-binding sites on human platelets we compared the effect of trace amounts of thrombin with that of ADP. In preliminary experiments we determined that a low concentration of thrombin (0.05 U ml^{-1}), is blocked by hirudin (0.1 U ml^{-1}) in terms of thrombin's action on human fibrinogen. Thus, after exposure of platelets to thrombin, its enzymatic activity toward fibrinogen could be neutralised. After stimulation with thrombin (0.05 U ml^{-1}) and then inactivation with hirudin, we observed specific binding of ^{125}I-fibrinogen with remarkably similar characteristics to those seen after stimulation with ADP (Fig. 1). We estimate that 44,000 molecules of fibrinogen bound with the apparent dissociation constant of 1.8×10^{-7} M (Fig. 1, right-hand inset). Thus traces of thrombin can also induce mobilisation of platelet-binding sites for plasma fibrinogen, a process shown to occur after stimulation of human platelets with ADP[15]. Because thrombin is almost instantly detectable when blood vessels are wounded[16], our observation that traces of thrombin mobilise fibrinogen receptors in human platelets is important in the platelet-fibrinogen interaction.

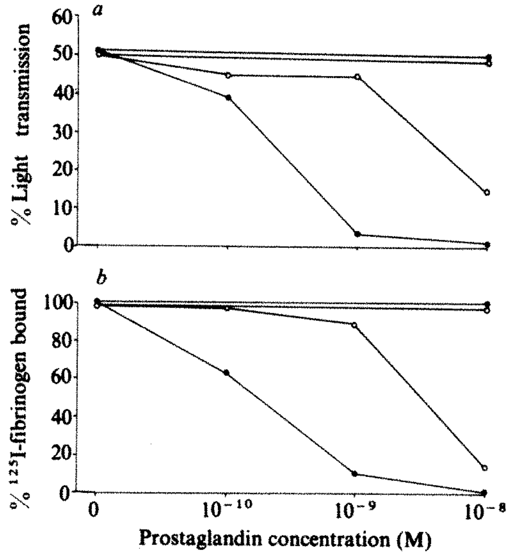

Fig. 2 Concentration-dependent effect of prostacyclin (lower two lines) and 6-keto PGF$_{1\alpha}$ (upper two lines) on binding of ^{125}I-labelled fibrinogen (*b*) and platelet aggregation (*a*) induced by ADP (●) and by thrombin (○). For platelet aggregation with ADP, fibrinogen (1.8 mg ml^{-1}) was added.

We next examined the possible regulatory role of prostacyclin in this interaction. Prostacyclin (1 nM) inhibited binding of ^{125}I-fibrinogen to human platelets stimulated with ADP, an effect that was paralleled by inhibition of ADP-induced platelet aggregation (Fig. 2). The dose–response curves for both inhibition of binding of ^{125}I-fibrinogen to platelets and inhibition of platelet aggregation were similar. Prostacyclin (10 nM) inhibited more than 80% of the binding of ^{125}I-fibrinogen to human platelets stimulated with thrombin (0.05 U ml^{-1}) and also inhibited platelet aggregation induced by the same concentration of thrombin. A 3-min pre-incubation was chosen in consideration of the relatively short lifetime of prostacyclin. However, the inhibitory effect on fibrinogen-binding lasted much longer, up to 60 min after the addition of prostacyclin to platelets.

Our experiments demonstrate that prostacyclin inhibited both ADP- and thrombin-induced mobilisation of binding sites for fibrinogen, which was paralleled by the inhibition of platelet aggregation and suggests a close association between mobilisation of fibrinogen-binding sites and platelet function. Prostacyclin raises the intraplatelet level of cyclic AMP[3,4], which can also be attained by PGD_2 (ref. 17). Indeed, PGD_2 (10 nM) also inhibited mobilisation of binding sites for fibrinogen in human platelets (results not shown). These findings suggest that mobilisation and availability of binding sites for fibrinogen are controlled by the cyclic AMP level. A decrease in cyclic AMP, resulting from ADP and thrombin action[18], promotes mobilisation of fibrinogen-binding sites, important for the subsequent formation of a platelet fibrin plug. An increase in cyclic AMP in response to prostacyclin prevents unmasking of fibrinogen-binding sites.

Although the nature of binding sites for fibrinogen on human platelets is unknown, their number after mobilisation by ADP and thrombin (50,000 and 44,000) is within the range of the 40,000 surface fibrinogen molecules per platelet determined from saturation binding of antifibrinogen Fab fragments[7]. The values established in our experiments are close to the reported number of binding sites induced by ADP[19]. We observed that sites mobilised by ADP and by thrombin are specific, saturable and of high affinity. The process responsible for mobilisation of binding sites of such affinity for fibrinogen can be related to changes in platelets induced by ADP and by a low concentration of thrombin. The changes include transformation from discoid to spherical, protrusion of several pseudopods and distension of the lumen of surface-connecting canaliculi[20]. These changes are accompanied by a decrease in adenylate cyclase activity and by the increase and redistribution of binding sites for lentil lectin demonstrated by Feagler *et al.*[14]. Increased and transient association of fibrinogen with human and rabbit platelets undergoing ADP-induced shape change was observed by Mustard *et al.*[21].

Note that the platelet-fibrin plug formed at the site of a vascular wound does not expand beyond its immediate zone or occlude the lumen of the injured vessel[5]. The inhibitory effect of prostacyclin produced by the vessel wall on mobilisation of fibrinogen-binding sites on human platelets may limit the size of the platelet-fibrin plug *in vivo*. Furthermore, this inhibitory action of prostacyclin can explain its protective effect on platelet consumption in extracorporeal circulation[22] because the interaction of platelets with foreign surfaces requires the presence of fibrinogen[23]..

Bovine thrombin, hirudin, adenosine, AMP, ADP and ATP were purchased from Sigma. Prostacyclin 6-keto $PGF_{1\alpha}$ and PGD_2 were obtained from Drs E. Nishizawa and J. Pike, Upjohn Co. This work was supported by the USPHS grants HL-24119 and HL-08339 and by the Medical Research Service of the Veterans Administration.

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A Con A-stimulated T-cell hybridoma releases factors affecting haematopoietic colony-forming cells and B-cell antibody responses

John W. Schrader, Bernd Arnold & Ian Clark-Lewis

The Walter and Eliza Hall Institute of Medical Research, Post Office,
Royal Melbourne Hospital, Victoria 3050, Australia

The major role of the thymus-dependent class of lymphocytes (T cells) seems to be regulatory. With the exception of cytotoxic T cells, T-cell subsets act by modulating the function of other T and B lymphocytes[1,2] and macrophages[3], and also the progenitors of erythrocytes, monocytes and myeloid cells[4–7]. Most of these regulatory effects are mediated by humoral factors, but despite convincing evidence that T cells are essential for the production of such factors, in many instances it has been difficult to demonstrate conclusively that the T cells actually synthesise the factors[7]. Factors stimulating the formation of myeloid colonies in agar can be produced by a T-cell lymphoma, EL4 (ref. 8), and by a hybridoma formed by the fusion of a T-cell lymphoma with pokeweed mitogen-stimulated spleen cells, although in this case it was not possible to demonstrate directly that the non-tumour parent had been a T cell[9]. Here we report that a cloned T-cell hybridoma can be induced to release factor(s) stimulating both the growth of haematopoietic colony-forming cells and the B-cell response to antigen. Incubation of the hybridoma with the T-cell mitogen concanavalin A (Con A), was required for the production of these activities, which were detectable 24 h after addition of the Con A. These activities were not produced when the parental tumour (BW5147) was incubated with Con A.

The hybridoma was formed as described elsewhere[10] by fusing a hypoxanthine–aminopterin–thymidine-sensitive variant of a T-cell lymphoma (BW5147) with blast cells isolated from the lymph nodes of mice immunised with keyhole limpet haemocyanin (KLH)[11]. Hybridomas resulting from the fusion were initially cloned by limiting dilution and the Con A-reactive clone,

Table 1 The colony-stimulating activity of supernatants of hybridoma cells stimulated with Con A

Supernatant from cultures containing:	Colonies per dish
T-cell hybridoma cells	0, 0
T-cell hybridoma + Con A (0.5 $\mu g\ ml^{-1}$)	83, 90
T-cell hybridoma + Con A (5 $\mu g\ ml^{-1}$)	160, 126
TKA	0, 0
TKA + Con A (0.5 $\mu g\ ml^{-1}$)	0, 0
TKA + Con A (5 $\mu g\ ml^{-1}$)	0, 0
Spleen cells + Con A (5 $\mu g\ ml^{-1}$)	81, 70

Hybridoma cells (3×10^5) were incubated for 24 h in 2 ml of Dulbecco's modified Eagle's medium (DMEM) supplemented with 2-mercaptoethanol (5×10^{-5} M), L-asparagine (3×10^{-4} M), glutamine (2.8×10^{-3} M), 10% fetal calf serum (FCS) and Con A (Pharmacia) at the indicated concentrations. Supernatants were prepared by centrifugation and 0.1-ml aliquots placed in 35-mm plastic Petri dishes to which were added 1-ml aliquots of 0.3% Bacto-agar (Difco) in the medium described above minus 2-mercaptoethanol; each ml contained 5×10^5 viable nucleated cells from the femoral shafts of CBA/H/Wehi mice. Also included were control dishes containing 0.1 ml of the supernatants of cultures in which the T-cell lymphoma TKA was incubated with Con A, and control dishes containing 0.1 ml of the supernatant of 48-h cultures of 50×10^6 C57BL/6 spleen cells in 10 ml of medium with Con A (5 $\mu g\ ml^{-1}$). Colonies were counted with a dissecting microscope on day 7. Morphology of colony cells was determined by plucking individual colonies and then either drying and staining them with aceto-orcein, or smearing and staining them with Giemsa stain.

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designated 1.23, was then recloned in agar. It expressed both the Thy 1.1 antigen of the AKR-derived BW5147 tumour and the Thy 1.2 antigen of the CBA lymph-node donor, and possessed 79 chromosomes.

The hybridoma 1.23 did not bind KLH, as determined with fluorescein-conjugated KLH on the fluorescence-activated cell sorter (B.A., F. L. Battye and J. F. A. P. Miller, unpublished observations). Furthermore, the addition of KLH, either in soluble form or presented on peritoneal exudate cells, did not result in the release of regulatory factors. Thus, we have no evidence that the hybridoma 1.23 has receptors for, or is sensitive to, KLH.

We were aware of the reported instability of T-cell hybridomas. Therefore, we have been careful to store multiple aliquots of both the original clone and subsequently recloned sublines in liquid nitrogen, always recloning in agar after thawing. Experience has shown that the chromosome number is not stable and we have observed chromosome loss in continuously passaged sublines, in some cases without loss of the ability to release regulatory activities. We are exploring the possibility that chromosomal loss may result in functional variants that will aid in the investigation of the number of molecules responsible for the observed biological activities and the mechanism of their release.

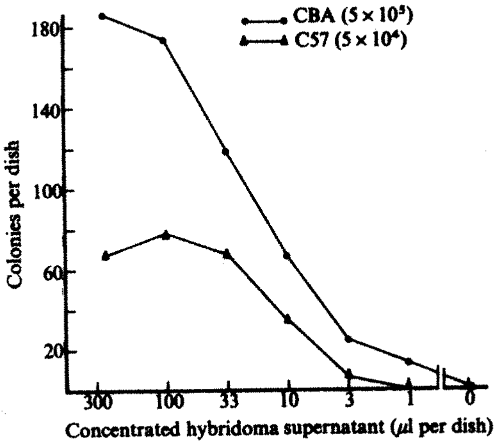

Fig. 1 The colony-forming response of bone marrow from CBA and C57BL/6 mice to supernatants of cultures of Con A-stimulated hybridoma cells. Agar cultures were carried out as described above and contained either 5×10^5 viable nucleated CBA bone marrow cells or 5×10^4 C57BL/6 bone marrow cells. The hybridoma supernatant was prepared from cells cloned twice in agar and grown in DMEM containing 10% FCS and 5×10^{-5} M 2-mercaptoethanol, by incubating 50×10^6 cells in 50 ml of medium without FCS, together with 5 μg ml^{-1} of Con A (Pharmacia). After 24 h the cell-free supernatants were collected by centrifugation and concentrated ninefold in an Amicon stirred cell with a 10,000 molecular weight cutoff membrane (Amicon YM10); they were sterilised by filtration through a 0.45-μm filter (Millipore).

Table 1 shows an experiment demonstrating Con A-dependent secretion by hybridoma 1.23 of factor(s) stimulating the growth of colonies in semi-solid cultures of normal CBA bone marrow cells. Differences were noted in the effects of supernatants of stimulated 1.23 cells on bone marrow cells from two strains of mice. Figure 1 demonstrates the effects of various amounts of concentrated supernatant from a culture of Con A-stimulated hybridoma cells, on the numbers of colonies in cultures of bone marrow cells from two mouse strains, CBA (plated at 5×10^5 per dish) and C57BL/6 (plated at 5×10^4 per dish). In both cases, colony numbers varied linearly with the number of cells cultured (data not shown). As shown in Fig. 1, in these culture conditions, the frequency of colony-forming cells was higher (by a factor of 4) in C57BL/6 bone marrow than in CBA bone marrow. Also, C57BL/6 colonies contained either macrophages or granulocytes or both, whereas up to 30% of the CBA colonies contained large multinucleate cells resembling megakaryocytes, together with blast cells and cells with annular or lobed nuclei, the remaining colonies consisting of blast cells and granulocytes or mixtures of granulocytes and macrophages.

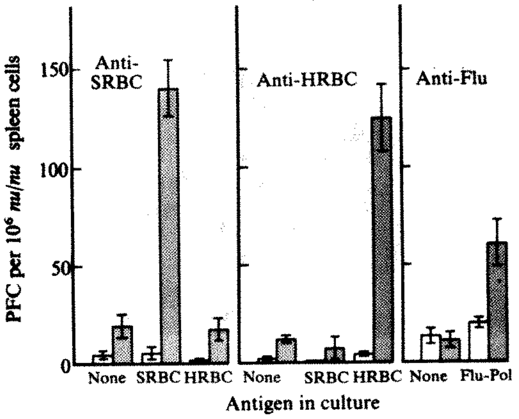

Fig. 2 The effect of supernatants of Con A-stimulated hybridoma cells on the B-cell antibody response to three antigens. Microwell cultures of *nu/nu* spleen cells containing no antigen, sheep red blood cells (SRBC) (10^6 per well), horse red blood cells (HRBC) (10^6 per well) or fluorescein-conjugated polymerised flagellin (Flu-Pol) (20 ng per well)[16], were cultured either with (hatched columns) or without (open columns) hybridoma supernatant (20%) for 4 days. Cultures incubated with erythrocyte antigens were divided in half and assayed against both SRBC and HRBC for plaque-forming cells (PFC). Cultures containing Flu-Pol were divided in half and assayed against fluorescein-bearing SRBC[16], and SRBC, the difference in PFC numbers being shown as Flu-specific PFC. Results are expressed as the mean ±s.e.m. of eight replicate cultures. The direct addition of Con A to the cultures, either with or without antigen, had no effect (data not shown). The cultures, in Linbro flat-bottomed trays (76-003-05), contained 10^6 viable nucleated BALB/c *nu/nu* spleen cells in DMEM (0.25 ml) supplemented with 5% FCS, 5×10^{-5} M 2-mercaptoethanol, 10 mM HEPES, 2.8×10^{-3} M glutamine and 3×10^{-4} M asparagine. The hybridoma supernatant was prepared and concentrated as described in Fig. 1 legend from cells cloned twice in agar.

Similar results were obtained using medium conditioned by Con A-stimulated spleen cells. Furthermore, there are other indications of differences between various haematopoietic progenitor cells in these two strains. Thus, the granulocyte–macrophage colony-forming cells in C57BL/6 bone marrow are twice as frequent and are much more sensitive to the colony-stimulating factor elaborated by endotoxin-stimulated mouse lung than are those from CBA mice[12]. Furthermore, in cultures stimulated by pokeweed mitogen-stimulated spleen cells, the frequency of mixed or erythroid colonies in fetal liver[13] or adult bone marrow (G. R. Johnson, in preparation) is markedly higher in the CBA than the C57BL/6 strain. Future study should determine both the nature of the colony-forming cells stimulated by the hybridoma supernatants and whether separate molecules are responsible for the growth of the different colonies.

Figure 2 shows the results of an experiment demonstrating that the hybridoma 1.23 supernatant affected B lymphocytes, allowing cultures of spleen cells from congenitally athymic (*nu/nu*) mice to develop an antibody response to the thymus-dependent antigen, sheep erythrocytes. Both the hybridoma supernatant and the antigen were required for a response. This effect of the supernatant was similar to that achieved with medium conditioned by Con A-stimulated spleen cells, and seems to be functionally equivalent to the T-cell replacing factor (TRF) described by Schimpl and Wecker[14]. This effect, although requiring the presence of antigen, was non-antigen specific. Thus, the response to a second heterologous erythrocyte, horse erythrocyte, was also enhanced (Fig. 2). The results show that cross-reaction between these antigens was not detected. Furthermore, the response to a third, quite unrelated antigen, fluorescein-conjugated polymerised flagellin (Flu-Pol) was also enhanced. The enhancement of the thymus-independent response to this antigen is consistent with the notion that the hybridoma supernatant is acting subsequent to B-cell activation, amplifying the antibody response in a non-antigen specific way[14,15].

These observations are interesting from several points of view. First, the availability of a clonal cell line offers great advantages for the reproducible, large-scale preparation of material for biochemical analysis. Preliminary experiments suggest that the hybridoma supernatants also support the *in vitro* proliferation of T lymphocytes and pluripotential stem cells, indicating that several different factors could be involved. It will be important to determine how many molecular species are involved in these activities and, if there are more than one, what relationship there may be between them. In this connection, it is of interest that analyses of medium conditioned by Con A-stimulated spleen cells, the biological activity of which seems to parallel in all respects that of the hybridoma supernatants, show that the B-cell effect and the colony-stimulating activities are separable biochemically, indicating the presence of at least two types of regulatory molecule (I.C.-L. and J.W.S., unpublished data).

Second, the observation that a cloned cell line with definite T-cell markers produces regulatory factors when stimulated with Con A in the same manner as do spleen cell suspensions, indicates that T cells themselves can produce these factors. Third, the demonstration that supernatants derived by stimulation of a cloned cell line enhance antibody responses to several antigens (Fig. 2), is against the notion that the 'T-cell replacing' activity produced by Con A-stimulated spleen is not really non-antigen specific, but is due to the release of a mosaic of antigen-specific T-cell factors on polyclonal stimulation of T cells.

Finally, functional T-cell hybridomas have proven more elusive than their B-cell counterparts. Studies of the conditions required for activation of this hybridoma by Con A may aid in defining conditions for the optimal induction and function of T-cell hybridomas. Furthermore, assays for the release of soluble factors such as those described here may prove useful as screening procedures for the detection of stimulation of T-cell hybridomas by specific antigen.

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Development of specific suppressor cells in hypoinsulinaemic mice

Wlodzimierz Ptak, Maria Rewicka & Maria Kollat

Department of Experimental and Clinical Immunology, Institute of Microbiology, Copernicus Medical Academy, 31-121 Kracow, Poland

Mice and rats injected with alloxan or streptozotocin develop permanent diabetes, characterised by deficient insulin production. It has been demonstrated that hypoinsulinaemia in mice leads to significant loss of lymphatic tissue[1,2], and these diabetic animals cannot develop contact sensitivity[1] or efficient graft rejection[3,4]. Administration of insulin partially restored these responses[1,4] and also caused an increase in the weight of the thymus and spleen[1]. Similar suppression of T cell-dependent phenomena has been observed in surgically pancreatectomised rats[5]. Lymphocytes of these hypoinsulinaemic animals show significantly decreased *in vitro* responses to plant lectins[2,6,7] and generate only low levels of cytotoxic effector cells[2,7]. We previously showed that cells of normoglycaemic oxazolone-sensitised mice cannot transfer significant contact sensitivity reactions into diabetic recipients[1] indicating that the milieu of hyperglycaemic insulin deficient animals cannot support all the activity of immune T cells. By mixing immunised T cells from control and diabetic mice and transferring the mixtures into normal recipients we now show that the non-supportive milieu in diabetic animals may be due to active suppression rather than to athrepsis.

CBA and DBA/2 inbred male mice were rendered diabetic by intravenous (i.v.) injection of alloxan (75 mg per kg). Only mice with blood glucose levels exceeding 400 mg per 100 ml and showing no signs of diabetic disease were incuded in the experiments[1]. Normoglycaemic mice were sensitised by application of 0.1 ml of 7% picryl chloride (PCL) or 3% oxazolone (OX) to the skin of the clipped abdomen[8]. Diabetic mice were sensitised 4 days after alloxan administration with lower doses of PCL (3%) and OX (1%) than normal mice because higher doses tend to be toxic in these mice. Normal mice respond similarly to both antigen doses[8,9]. Four days later single cell suspensions were prepared from the spleens and peripheral lymph nodes of the diabetic and control animals. In some experiments cells of diabetic CBA mice were treated with anti-Thyl serum and complement[10] or T cells were isolated with the help of nylon wool columns[11]. Cells from sensitised normoglycaemic animals (4×10^7) were injected i.v. into normal recipients. Some recipients also received 2×10^7 cells from diabetic mice which had or had not been previously immunised. Immediately after transfer the mice were challenged on the ear with 1% PCL or OX in olive oil and the increment of ear thickness measured after 24 h[8].

The results of such an experiment (Table 1) shows that nylon non-adherent Thy 1 positive cells from immunised diabetic mice abrogate in an antigen-specific way the ability of normal immune T cells to transfer contact sensitivity. Thus, a lack of insulin changes an otherwise immunogenic sensitising procedure into one which preferentially activates suppressor T cells.

The induction of specific suppressor cells by antigen in hypoinsulinaemic environment could be explained in a number of ways. One possibility is that distinct subpopulations of T lymphocytes show selective suspectibility to high glucose levels (or high osmotic pressure). This interpretation, although not disproved, seems unlikely since T helper cells function quite well *in vitro* at glucose concentrations much higher than those present in diabetic animals[12]. Another possibility, and the one we favour, is that the activity of lymphocytes is directly influenced by the insulin level. The effects of insulin may depend on its overall action on metabolic pathways which could explain the general loss of lymphatic tissue in diabetic animals. Some

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Table 1 Suppression of passive transfer of contact sensitivity (CS) by lymphocytes of diabetic animals

Lymphocytes of diabetic animals, specificity and treatment	CS reaction ($\times 10^{-3}$ cm) PCL	OX
No cells (−ve control)	1.7 ± 0.25	2.4 ± 0.36
No cells (+ve control)	6.5 ± 1.04	8.3 ± 1.33
PCL	3.0 ± 0.63	8.4 ± 0.87
OX	7.2 ± 0.74	4.9 ± 0.56
Not sensitised	7.0 ± 0.37	8.0 ± 0.60
PCL, purified T cells	2.2 ± 0.41	ND
PCL, anti-Thy 1 treated	7.8 ± 0.51	ND
OX, purified T cells	ND	4.0 ± 0.34

Normal CBA recipients were injected with 4×10^7 cells of normal animals sensitised with PCL or OX 4 days previously, mixed with 2×10^7 cells from diabetic animals which had (or had not) been sensitised with either PCL or OX 4 days before transfer. In some experiments cells from hyperglycaemic mice were purified on nylon wool columns (purified T cells) or treated with anti-Thy 1 serum and C′ before being injected to the hosts. This serum killed >90% nylon column purified T cells. Mice were challenged on the ears with the homologous hapten. Each group consisted of six to eight mice. Similar results were obtained with DBA/2 mice. Results are given as mean ±s.d. +ve control refers to animals which received immune normal cells only; −ve control refers to mice which received no injection of cells (nonspecific ear swelling).

selectivity of insulin action, however, argues against this simple scheme. It has been found that insulin augments the *in vitro* cytotoxicity of sensitised lymphocytes[13] and insulin receptors have been demonstrated on cytotoxic lymphocytes[14] as well as on cells activated with concanavalin A[15]. The majority of blood lymphocytes do not express demonstrable insulin receptors which suggests that subsets of T lymphocytes may differ in their expression and thus may be selectively sensitive to insulin deficiency.

A large body of data has accumulated which suggests that cytotoxic effector cells and suppressor T lymphocytes share several characteristics[16,17], including the expression of hormone receptors[14], which are not shared by helper cells. If these similarities extend to insulin receptors we would then postulate a direct effect of the lack of insulin on the expression of suppressor cell activity. Since the lack of insulin increases suppressor cell activity, as demonstrated above, our results could suggest that the presence of insulin is an important factor in damping down normal suppressor cell activity. This postulate would predict that subjects with deficient levels of insulin would be more susceptible to infection because of hyperactivity of their suppressor cells. However, since the activation of suppressor cells has been shown to be the result of a complex series of interactions between T cell sets and since it has also been shown that the activity of cells outside of the suppressor circuit can influence the levels of suppression which become manifest after immunisation[18], our results do not indicate directly that the suppressor cell itself is the target of insulin mediated control. Further studies using antisera which define functionally distinct T cell subsets are required before we can definitely say how the lack of insulin leads to increased suppressor cell activity. We are also attempting positive selection studies, using insulin columns to directly test the nature of the cells which binds to the columns. The fact that insulin has been shown to increase the activity of sensitised killer lymphocytes[2,7], and that its lack appears to increase the level of suppressor cell activity should not be taken as evidence that the putative insulin receptors of these two sets of T cells are in any way different. The possibility that a single agonist can have two opposing effects depending upon dose, should be taken into consideration. The observations, however, that discrete subpopulations of T lymphocytes carry receptors for various hormones (for example, growth hormone[19] or histamine and adrenaline[20]) make our hypothesis of selective expression of insulin receptor tenable and testable.

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Phototransduction in the fly compound eye exhibits temporal resonances and a pure time delay

Andrew S. French

Department of Physiology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

Photoreceptor cells in both vertebrates and invertebrates respond to a flash of light with a slow graded change in membrane potential, which is generally depolarising in invertebrates and hyperpolarising in vertebrates. Although some of the early photochemical and biochemical stages of the transduction process have been elucidated in both cases[1,2], these reactions are fast compared with the time course of the electrical response, which is typically hundreds of milliseconds long. To explain this slow response in the eye of *Limulus*, Fuortes and Hodgkin[3] proposed a mechanism in which the light signal passes through a cascade of simple low pass filters. The model was later defined more specifically in terms of a chain of chemical reactions linked together through products and reactants[4], and has been used with small modifications and different numbers of stages to account for the behaviour of various vertebrate and invertebrate photoreceptors including the fly compound eye[5,6]. I have now obtained evidence that phototransduction in the fly in small signal conditions involves underdamped resonance behaviour and a significant pure time delay, neither of which can be accounted for by the conventional cascade model.

The frequency response function is an alternative means of characterising the dynamic behaviour of a photoreceptor, and yields an identical description to that given by the response to a brief flash of light if the photoreceptor behaves linearly. In practice the frequency response method is more useful than the flash method because it allows a photoreceptor to be examined in small signal conditions with relatively small light fluctuations where behaviour is linear. When the white noise stimulation technique is used to measure the frequency response function it is possible to characterise a photoreceptor very efficiently and simultaneously to measure the linearity and signal to noise level of the system through the coherence function[6].

In the frequency domain the long-delayed response of photoreceptors becomes a phase lag which increases with frequency and there is also a substantial amplitude attenuation with increasing frequency. The Fuortes and Hodgkin model predicts these effects but also predicts a maximum phase lag asymptote the value of which is given by 90° multiplied by the number of stages in the cascade. This asymptotic phase lag has never been demonstrated clearly and most published frequency response functions for photoreceptors show a phase lag which is

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unbounded with increasing frequency. In previous investigations of the fly photoreceptor system[6,7] we tried to find a phase lag asymptote and tentatively suggested that it occurs at a lag of 450°, characteristic of a five-stage cascade. However, examination of the coherence functions which we obtained for this preparation reveals that in the frequency range where the asymptote should appear, which is above 100 Hz in the fly, the signal to noise ratio is so low that there is little statistical confidence in the measurements. Because this problem arises from the inherent noise level of the photoreceptor, the same difficulty will apply to the flash response measurement, but it will be the accuracy of the most rapidly changing regions of the response which are unreliable.

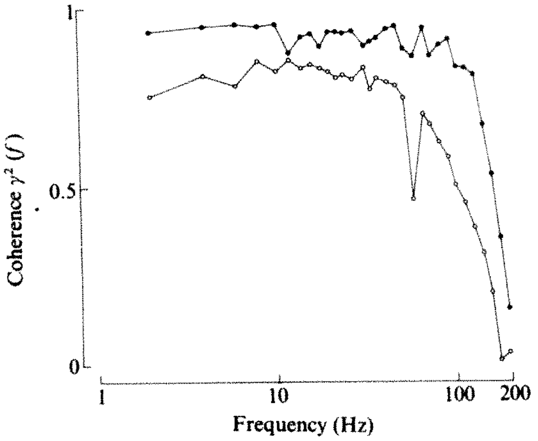

Fig. 1 Coherence function, $\gamma^2(f)$, for phototransduction in a fly photoreceptor cell. The open circles are from an experiment conducted with the conventional white noise technique using 25 sequences of noise, each lasting 512 ms. The input and output data were sampled at 1-ms intervals and processed using the fast Fourier transform. The filled circles are from the same experiment but with each of the 25 sequences being presented 10 times and the average response taken. The improvement in signal to noise ratio may be quantified by use of the expression $\gamma^2(f) = 1/(1 + N(f)/S(f))$, where $S(f)$ is the signal power and $N(f)$ is the noise power at frequency f[11]. Applying this equation at 100 Hz gives more than a fivefold improvement in signal to noise power ratio. Solid lines are drawn through the data points for visual clarity. The large dip in the unaveraged data is at 60 Hz, the a.c. power frequency, and represents unavoidable experimental pick up. It is also reduced substantially by the averaging process because it is uncorrelated with the stimulus.

I repeated the frequency response function measurements on the fly photoreceptor system using an averaging technique to improve the signal to noise ratio. The technique consists of stimulating the eye with repeated bursts of pseudo-random noise patterns of light intensity while averaging the responses to identical patterns. This averaging reduces the uncorrelated noise from the response and the averaged signal is processed as if it were the response to a single presentation of the random input. The experiments were conducted on the R1-6 photoreceptors of the compound eye of *Phormia regina* using a green light-emitting diode with peak output at 560 nm. Intact flies were mounted in wax and intracellular recordings of membrane potentials were made by glass microelectrodes filled with 3 M KCl and having resistances in the range 50–100 MΩ. The microelectrode was advanced through the back of the eye and a platinum ground electrode was placed in the thorax. Details of the experimental procedures and the averaging technique are given elsewhere[8,9].

Figure 1 shows a comparison of the coherence function measured for a photoreceptor with and without the averaging technique. There is a substantial improvement in the signal to noise ratio, particularly in the critical frequency region of 100–200 Hz where the phase lag asymptote should appear. Figure 2 shows the frequency response function between light fluctuations and membrane potential fluctuations for the same experiment as Fig. 1. The improved signal to noise ratio produces a smooth frequency response function to 200 Hz, which compares favourably with our published data[6,7].

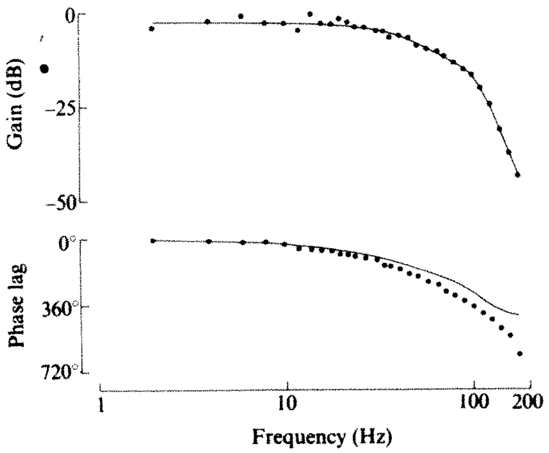

Fig. 2 Frequency response function between light fluctuations and fluctuations in membrane potential in the same photoreceptor cell as for Fig. 1. The mean current through the light emitting diode was 5.6 mA which gives a peak response of about 10 mV in a dark adapted photoreceptor. The mean intensity was modulated to a depth of 75% by the pseudo-random noise stimulus. The solid lines are the predicted response from a linear system consisting of a cascade of two first order poles with time constants of 1.13 and 1.56 ms and two second order poles with time constants of 3.83 and 1.49 ms and damping factors of 0.79 and 0.25, respectively. The phase data points fall progressively below the predicted response with increasing frequency and the difference between these two sets of values is shown in Fig. 3.

A low pass frequency response function can be factorised into an expression containing integrating terms, $(j\omega)^{-1}$, first order poles, $(1 + j\omega\tau)^{-1}$, and second order poles $(1 + 2j\zeta\omega\tau + (j\omega\tau)^2)^{-1}$, where $j = \sqrt{-1}$, ω is natural frequency, τ represents time constants, and ζ represents damping factors[10]. Integrating terms give rise to a constant phase lag of 90° at all frequencies and so were not considered further because of the reliable low frequency phase asymptote of 0°. The Fuortes and Hodgkin model would produce a series of first order poles, with each stage contributing a single time constant. Second order poles represent underdamped or resonant behaviour for $\zeta < 1$, or can be factorised simply into two first order poles for $\zeta > 1$. Pure time delay may also be present in a linear system and it produces a phase lag equal to the product of the delay and the frequency, without affecting the gain of the response.

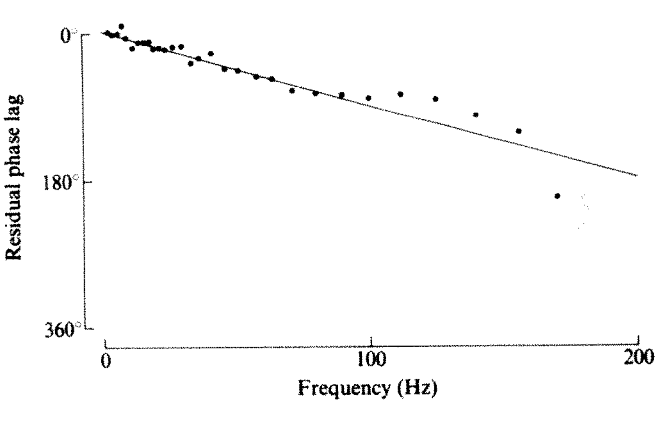

Fig. 3 Difference between the experimental phase lag of Fig. 2 and the predicted phase lag for the linear system which fits the gain data. The residual phase lag has been plotted on linear axes and fitted with a straight line by linear regression. The slope of the straight line corresponds to a delay of 2.48 ms and the linear regression coefficient is 0.95.

Frequency response functions of the type illustrated in Fig. 2 were fitted by an iterative minimum mean square error technique in which an initial estimate of the number and types of poles and their time constants was made. Then a computer program varied each component up and down in value while computing total square error between the estimate and the experimental data. The varied values producing the smallest error were then used as new estimates while the size of the variation was reduced and the process repeated until the variation was below 1%. The fitting process was applied to the gain portion of the response because this is independent of any delay. Initial attempts to fit the experimental data with first order poles were unsuccessful compared to the accuracy with which the data could be fitted using first and second order poles. The solid line in Fig. 2 is the fit produced by two first order poles and two second order poles. This model also gave the best fit to the data from 12 separate experiments, with average time constants and damping factors of: $\tau_1 = 0.92$ ms, $\tau_2 = 1.15$ ms, $\tau_3 = 3.28$ ms, $\zeta_3 = 0.88$ and $\tau_4 = 1.41$ ms, $\zeta_4 = 0.35$. The model did not account adequately for the phase data, which always lagged further than the prediction at higher frequencies. Because the only behaviour that could explain this discrepancy is a pure time delay the difference between the predicted and actual phase data was plotted on linear axes and fitted with a linear regression line, as illustrated in Fig. 3. This produced a good fit in all experiments with a mean delay value of 2.85 ms and a mean correlation coefficient of 0.93.

These results show that the linear cascade model is inadequate to account for phototransduction in the fly and a model must be constructed which predicts the underdamped behaviour and the pure time delay.

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Ca^{2+} transmembrane fluxes and nerve growth factor action on a clonal cell line of rat phaeochromocytoma

Gary Landreth, Paul Cohen* & Eric M. Shooter

Department of Neurobiology, Stanford University School of Medicine, Stanford, California 94305

Nerve growth factor (NGF) is a polypeptide hormone which has a fundamental role in the development and maintenance of sympathetic and some sensory neurones[1,2]. The primary event by which NGF exerts its actions on these neurones probably involves the recognition of specific membrane-located receptors[3–5]. A clonal cell line of rat phaeochromocytoma, PC12, responds to NGF by cessation of division and expression of several properties characteristic to mature sympathetic neurones, including neurite outgrowth[6,7]. These cells also possess cell-surface NGF receptors, a fraction of which are occupied at NGF concentrations which elicit NGF-specific responses in these cells (G.L. and E.M.S., unpublished results). The manner in which binding of NGF to its receptor is translated into the subsequent morphological and biochemical events is not yet known but is being investigated. One possible transducing mechanism which has been suggested involves transient changes of intracellular cyclic AMP levels[8,9]. The divalent cation Ca^{2+} has been proposed as a second messenger in the mechanism of action of peptide hormones such as insulin[10,11], which also initiates its action through membrane-bound receptors. Here, we have investigated the hypothesis that the mechanism of NGF action on PC12 cells may involve modulation of calcium transmembrane fluxes. The evidence shows that NGF has no significant influence on calcium fluxes as measured by uptake and efflux rates of ^{45}Ca in PC12 cells.

PC12 cells were grown in Dulbecco's modified Eagle's medium containing 4.5 mg l^{-1} glucose, supplemented with 10% fetal calf serum and 5% horse serum. For the measurements of calcium fluxes in single cell suspensions of PC12 cells, the culture medium was removed and the cells washed three times with 10 ml of Tris-buffered Gey's solution (TBG), *p*H 7.4, containing 1.5 mM Ca^{2+} and 0.1% bovine serum albumin. The cells were centrifuged for 3 min at 500*g*, the supernatant discarded and the pellet resuspended in the same buffer at a final concentration of 10^6 cells ml^{-1}. Cell viability was evaluated before and after the calcium flux measurements by trypan blue exclusion and was always 85–95%. The cells were preincubated for 40–60 min with gentle shaking at 37 °C in the absence of ^{45}Ca for the uptake experiments or in the presence of ^{45}Ca for efflux experiments.

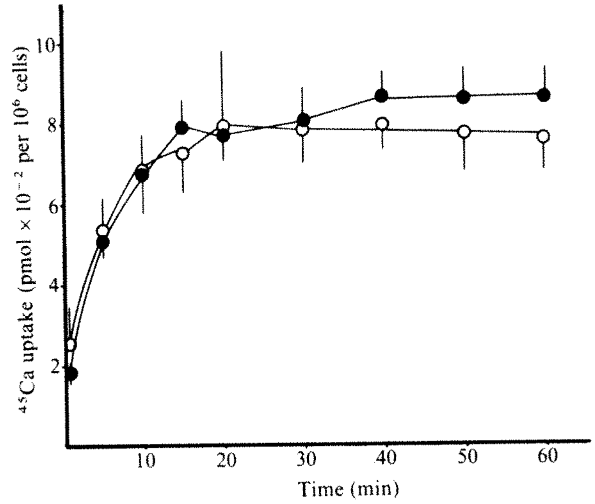

Fig. 1 ^{45}Ca uptake by PC12 cells. A suspension of PC12 cells (8 ml of a suspension of 10^6 cells ml^{-1}) was preincubated with gentle shaking for 40 min at 37 °C in TBG. At the end of this preincubation ^{45}Ca was added to one half of the sample to give a final specific activity of 30 μCi μmol^{-1}, and NGF was added to the remaining half of the sample together with ^{45}Ca to the same final specific activity. The ^{45}Ca content of the cells was determined by layering 0.1 ml of the cell suspension on the top of a two-step sucrose gradient (50 μl of 0.15 M and 150 μl of 0.3 M sucrose in a 400-μl Beckman microfuge tube), centrifuging for 30 s in the microfuge, rapidly freezing the tube and cutting off the section containing the cell pellet. The pellet in the tube tip was treated overnight in 0.4 ml of Protosol, scintillation fluid added and the radioactivity determined. Each point represents the mean (±s.d.) of three separate experiments. ●, Control cells; ○, cells incubated with 3 nM NGF.

The effect of NGF on ^{45}Ca uptake was measured on suspensions of PC12 cells (Fig. 1). The uptake reaction was initiated by the addition of ^{45}Ca alone or with 3 nM NGF. NGF had no effect on either the rate or magnitude of the ^{45}Ca uptake reaction. Similar results were obtained if NGF (3 nM) was added at the beginning of the preincubation period (data not shown).

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* Permanent address: Groupe de Neurobiochimie, Université P. et M. Curie, 96 B^d Raspail, 75006 Paris, France.

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Table 1 ^{45}Ca efflux from preloaded PC12 cells attached to a substratum

	Calcium preloading period (h)	Experiment	Radioactivity released (d.p.m. ^{45}Ca ml^{-1}) 30 min		60 min		*n*
a	1	1 Control	1,450 (±150)	$P<0.4$	1,590 (±130)	$P<0.4$	3
		NGF	1,620 (±230)		1,740 (±180)		3
		2 Control	1,060 (±120)	$P<0.5$	1,140 (±120)	$P<0.5$	4
		NGF	1,110 (±40)		1,160 (±90)		4
b	48	Control	1,140 (±150)	$P<0.4$	1,280 (±120)	$P<0.1$	3
		NGF	1,010 (±110)		1,100 (±80)		3

a, PC12 cells (log phase) were washed with TBG as described in the text and 3×10^6 cells were plated onto polylysine-treated 60-mm tissue culture dishes. ^{45}Ca was added immediately to a final specific activity of 1.3 μCi μmol^{-1} and cells were preincubated for 1 hr at 37 °C. The cells were rapidly washed three times in TBG or in TBG containing 3 nM NGF, 4 ml of the appropriate solution added to the dish and incubation carried out at 37 °C. After 30 or 60 min, 0.1-ml aliquots of the medium were taken in quadruplicate and radioactivity determined. In controls where there were no cells in the dish ~100 c.p.m. ml^{-1} were released from the dish. The data are presented as the mean of *n* samples; the figures in parentheses are standard deviations and *P* values were determined by Student's *t*-test. *b*, PC12 cells were grown (log phase) in Dulbecco's modified Eagle's medium to which 1.1 μCi μmol^{-1} ^{45}Ca was added 48 h before the cells were collected. The cells were removed from the tissue culture dish in the TBG containing the same concentration of ^{45}Ca, washed and then replated on polylysine-treated tissue culture dishes (60 mm, 3×10^6 cells per dish) in the same medium containing ^{45}Ca and preincubated for 1 h at 37 °C. The cells were then rapidly washed three times with calcium-free TBG or calcium-free TBG containing 3 nM NGF and 4 ml of this medium added to the dish. After 30 or 60 min, 0.1-ml aliquots of the medium were taken in quadruplicate and radioactivity determined.

The efflux of ^{45}Ca from suspensions of PC12 cells was measured after preloading the cells with ^{45}Ca for short (40 min) and long (25 h) periods. The efflux of ^{45}Ca from PC12 cells after a short preloading period is shown in Fig. 2. The exposure of the cells to 3 nM NGF produced no significant change in the rate of ^{45}Ca efflux. If the cells were preloaded for 25 h with ^{45}Ca, similar results were obtained when tested with 5 nM NGF or as much as 300 nM highly purified 7S NGF (ref. 12, data not shown). Both uptake and efflux studies were carried out with confluent cultures as well as those during log phase growth and identical results were obtained.

The efflux of ^{45}Ca from PC12 cells attached to tissue culture dishes was also investigated, as cell membrane-mediated events may be influenced by attachment of cells to a substratum. When PC12 cells (log phase) were preincubated with ^{45}Ca for 1 h on polylysine-treated tissue culture dishes (60 mm) and the efflux measured in the presence or absence of NGF (3 nM), no difference in the magnitude of the loss of ^{45}Ca was detected 30 or 60 min later (Table 1). Similarly, if the cells were preincubated for 48 h with ^{45}Ca and the efflux into calcium-free medium measured, NGF (3 nM) had no significant effect.

These results show that NGF has no demonstrable effect on transmembrane calcium fluxes, when measured with cells either in suspension or attached to a substratum. Efforts to alter intracellular calcium concentrations by addition of the calcium ionophore A23187 were unsuccessful because of the cytotoxic effects of the drug on PC12 cells in serum-free medium. When A23187 was tested in the presence of serum it had no effect on the NGF-mediated morphological differentiation. However, the binding of the drug to serum lipids rather than to the cell is a possibility which cannot be ruled out.

Schubert *et al.*[13] have recently suggested that NGF initiates its action on PC12 cells by causing a transient increase in intracellular cyclic AMP followed by a mobilisation and release of intracellular calcium stores. This conclusion was based on experiments which showed a small increase in the efflux of ^{45}Ca from NGF-treated cells compared with untreated cells[13]. The experiments reported here do not show an NGF-mediated alteration of ^{45}Ca efflux from cells in suspension when either low concentrations (3 nM) of NGF or high concentrations (300 nM) of 7S NGF were added. Moreover, the data shown in Table 1*b* were obtained in the same conditions as described by Schubert *et al.*[13] except that the NGF concentration was substantially lower. The lower NGF concentrations were chosen because the PC12 NGF receptors are fully saturated at 3 nM NGF (G.L. and E.M.S., unpublished observations), whereas half-maximal stimulation of neurite outgrowth occurs at 0.01 nM NGF (ref. 14). The binding of NGF to its cell-surface receptor does not require calcium[15]. It is noteworthy that Schubert *et al.*[13] used 800 biological units ml^{-1} of the 7S NGF complex (~300 nM), resulting in NGF concentrations several orders of magnitude above the physiologically effective concentrations of NGF for these cells. Even when tested at this concentration we were unable to detect any NGF-mediated alteration of ^{45}Ca efflux from suspensions of PC12 cells.

The hypothesis that NGF causes a rapid but transient increase in intracellular cyclic AMP preceding the calcium mobilisation

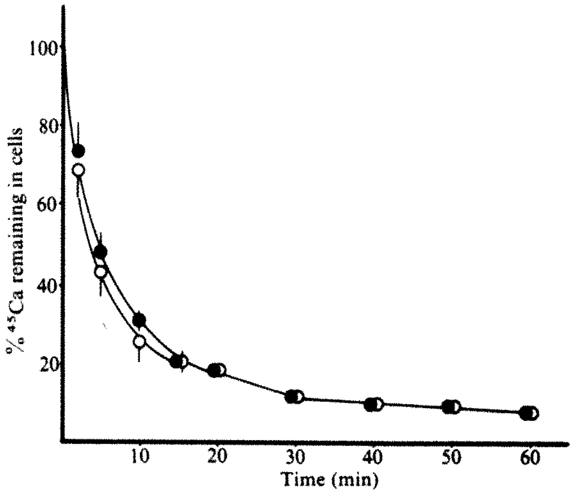

Fig. 2 ^{45}Ca efflux from PC12 cells. The time course of the ^{45}Ca efflux was measured after a suspension of PC12 cells (10^6 cells ml^{-1}) had been preincubated at 37 °C in TBG containing 5 μCi μmol^{-1} ^{45}Ca. After 60 min, 0.1-ml aliquots were taken and the cellular ^{45}Ca content determined as described in Fig. 1 legend. The radioactivity in the cells at this time was defined as 100%. The suspension was divided into two halves, each half centrifuged at 500*g* for 30 s, the supernatants discarded and the pellets resuspended with trituration in the same buffer but without ^{45}Ca. One half of the sample received no addition, and 3 nM NGF was added to the other half. The amount of ^{45}Ca remaining in the cells was evaluated by sampling 0.1-ml aliquots in triplicate at various times after transfer to the ^{45}Ca-free buffer. ●, Control cells; ○, cells incubated with 3 nM NGF. Each point represents the mean (±s.d.) of a series of three experiments, with each experiment consisting of triplicate samples. In each of the three individual experiments no significant difference between control and NGF-treated samples was observed.

in PC12 cells has also been questioned. Hatanaka *et al.*[16] could not demonstrate any alteration of intracellular cyclic AMP levels by NGF, in contrast to the findings of Schubert *et al.*[9,13]. The relationship of intracellular calcium concentrations and cellular differentiation is unclear[17], but it is possible that calcium may be involved in certain aspects of differentiation. However, in the light of the data presented here and those of others[16], the hypothesis that NGF action is mediated through cyclic AMP-dependent calcium mobilisation must be viewed with caution.

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Oscillation of $[Ca^{2+}]_i$-linked K^+ conductance in bullfrog sympathetic ganglion cell is sensitive to intracellular anions

K. Morita & K. Koketsu

Department of Physiology, Kurume University School of Medicine, Kurume 830, Japan

K. Kuba

Department of Physiology, Saga Medical School, Saga 840-01, Japan

The intracellular free Ca^{2+} ($[Ca^{2+}]_i$) regulates the K^+ conductance (G_K) of the many types of cell membrane[1–11]. The Ca^{2+} influx during an action potential activates this $[Ca^{2+}]_i$-linked G_K in most neurones[2–4,8–11]. In caffeine-treated sympathetic ganglion cells, however, Ca^{2+} released from an intracellular Ca^{2+} reservoir site analogous to the sarcoplasmic reticulum (SR) of the muscle (see ref. 12) causes activation of the G_K, which results in slow oscillatory hyperpolarisations (caffeine hyperpolarisation, C-hyperpolarisation)[11]. Such a release of Ca^{2+} linked to the G_K of the membrane seems important for understanding the role of the intracellular organelles in the control of membrane activities of a neurone. We report here the mechanism of the slow oscillatory hyperpolarisations recorded from the bullfrog sympathetic ganglion cell in Ringer solution. It is found that these hyperpolarisations are generated by a $[Ca^{2+}]_i$-linked G_K system and are highly sensitive to anions in an intracellular recording electrode, probably to intracellular anions.

Experimental procedures for intracellular recording from B-type neurones of paravertebrate sympathetic ganglia of the bullfrog (*Rana catesbeiana*) have been described previously[11,13,14]. Most experiments were carried out using microelectrodes filled with 1 M K_3-citrate (tip resistance 20–60 MΩ). In some experiments, electrodes were filled with 0.3 M K_2-sulphate, 3.0 M K-acetate or 3 M KCl (tip resistances 30–100 MΩ). Experiments were carried out at room temperature (20–25 °C).

When the ganglion cell was impaled with an electrode filled with 1 M K_3-citrate in Ringer, the resting membrane potential was relatively low (−40 to −50 mV), probably due to penetration injury[15]. Within a few minutes, the membrane began to hyperpolarise (repolarise), with an increase in the membrane resistance. In 10–20 min, the resting membrane potential and resistance reached a relatively high constant value (Fig. 1*Aa*, *b*). In this condition, the generation of an action potential was followed by a slow hyperpolarisation with a notable delay in many of the cells impaled (26 cells out of 38) (Fig. 1*Ab*). After a further 5–10 min, slow rhythmic hyperpolarisations appeared spontaneously in most cells (23 cells out of 38; Figs 1*Ac*, *B*, *C* and 2*Cc*). The amplitude (7.3 ± 0.8 mV (s.e.), $n = 23$) and time course (half-duration 1.6 ± 0.4 min) of these transient hyperpolarisations and the rate of their appearance (one every 6.4 ± 0.9 min, $n = 23$) remained almost constant as long as the resting potential was unchanged. Because, as will be described below, these hyperpolarisations seen in Ringer were very sensitive to intracellular anions, they will be called hereafter anion-sensitive hyperpolarisations (A-hyperpolarisations).

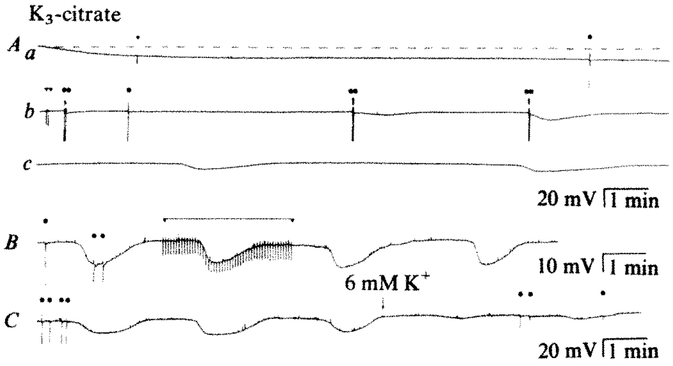

Fig. 1 Slow rhythmic hyperpolarisations (A-hyperpolarisations) recorded from three different cells. An action potential was induced by antidromic stimulation at times indicated by a closed circle (*A*–*C*). A hyperpolarising current pulse (0.3 nA, 250 ms) was applied through a recording electrode at times indicated by a triangle (*A*) or the current pulses (0.2 nA, 250 ms) were applied repetitively for the period indicated by the two triangles (*B*). *A*, Example of the time course of the membrane potential change after the penetration of the ganglion cell with an electrode filled with 1 M K_3-citrate. All the records (*a*–*c*) are continuous. The recording electrode was inserted immediately before the beginning of the record *a*. The spike component of the action potential is not shown (this is also true of the remaining figures where action potentials are shown). Note the appearance of an A-hyperpolarisation following the after-hyperpolarisation of an action potential and spontaneously occurring A-hyperpolarisations. *B*, The reductions of the membrane resistance and the amplitude of the after-hyperpolarisation of an action potential during the generation of an A-hyperpolarisation. *C*, Effects of an increase in the K^+ concentration in Ringer on A-hyperpolarisations.

The membrane resistance decreased dramatically during the generation of an A-hyperpolarisation (Fig. 1*B*). Raising the external K^+ concentration (6 mM) markedly reduced the amplitude of A-hyperpolarisations, although it depolarised the membrane only slightly (Fig. 1*C*). Conditioning membrane hyperpolarisations decreased the amplitude of the A-hyperpolarisation (Fig. 2*A*) and seemed to be reversed at a level at which the after-hyperpolarisation of an action potential was reversed (Fig. 2*B*). Thus, A-hyperpolarisations are apparently induced by an increased G_K of the membrane.

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As in the case of C-hyperpolarisations[11,13,14], A-hyperpolarisations were extremely sensitive to the external Ca^{2+} concentration ($[Ca^{2+}]_o$). Lowering $[Ca^{2+}]_o$ completely abolished A-hyperpolarisations (four cells) or decreased the rate of their appearance (three cells) in 3–5 min after the solution change (Fig. 2*C*), whereas the frequency of their appearance increased when $[Ca^{2+}]_o$ was raised. These effects of $[Ca^{2+}]_o$ were reversible. Furthermore, A-hyperpolarisations were sensitive to low temperature and inhibited by dantrolene Na (6.4 μM), as observed in the C-hyperpolarisation[11,13]. Thus, A-hyperpolarisations seem to be generated by a mechanism similar to that for C-hyperpolarisations, that is, increases in the G_K due to periodic rises in $[Ca^{2+}]_i$.

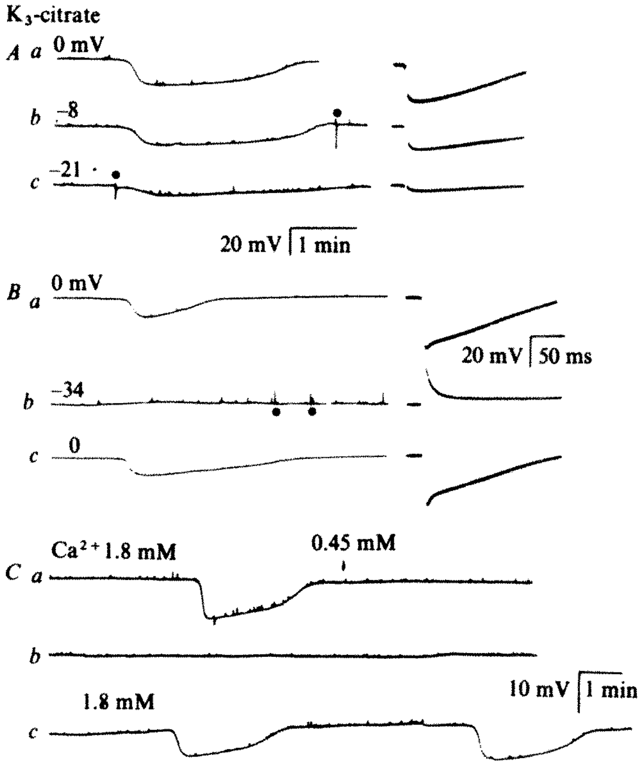

Fig. 2 *A*, *B*, Effects of conditioning membrane hyperpolarisations on the A-hyperpolarisation (left records) and the after-hyperpolarisation of an action potential (right records) in two cells. The level of membrane hyperpolarisation shown in the left side corresponds to both A-hyperpolarisations and after-hyperpolarisations of action potentials. An action potential was evoked by antidromic stimulation at times marked by closed circles in the left record of *Ab*, *Ac* and *Bb*. Action potentials are not shown in the left records of *Aa*, *Ba* and *Bc*. *C*, Effects of a reduction in the extracellular Ca^{2+} concentration in Ringer on A-hyperpolarisations. All the records are continuous. Note the disappearance of A-hyperpolarisations at a reduced Ca^{2+} concentration.

There is, however, a definite difference between the experimental conditions for recording A- and C-hyperpolarisations. The former were recorded by impaling a cell with an electrode filled with 1 M K_3-citrate in Ringer, whereas the latter were recorded with a 3 M KCl-filled electrode in a solution containing caffeine. This would imply that the species of an anion leaked out of the electrode into the cytoplasm, would determine the requirement for caffeine in the generation of oscillatory hyperpolarisations. Indeed, a very low dose of caffeine (0.1–1.0 mM) unable to induce C-hyperpolarisations augmented the amplitude and frequency of the A-hyperpolarisation, indicating that there was an increase in the apparent sensitivity of the ganglion cell to caffeine, when it was impaled with a K_3-citrate electrode.

Table 1 Effects of various anions in a recording electrode on the appearance of slow rhythmic hyperpolarisations at different caffeine concentrations

	Caffeine (mM)		
		0.5	1.0
K_3-citrate (1.0 M)	21/23 (91%)	25/25 (100%)	—
K-acetate (3.0 M)	11/16 (69)	14/18 (78)	18/18 (100%)
K_2-sulphate (0.3 M)	8/12 (67)	11/15 (73)	15/15 (100)
KCl (3.0 M)	4/18 (23)	11/22 (50)	22/22 (100)

Numerators are the number of cells in which rhythmic hyperpolarisations were observed, and denominators are the number of cells examined. In the experiments using electrodes filled with K-acetate, K_2-sulphate and KCl, the cells which showed rhythmic hyperpolarisations at a caffeine concentration of 3 mM were studied. When caffeine was absent rhythmic hyperpolarisations were recorded only in the cells which were penetrated with an electrode filled with 1 M K_3-citrate.

The ability of various anions to generate slow oscillatory hyperpolarisations was tested by penetrating a cell with an electrode filled with K-salts of various anions, and observing for each electrode the number of the cells which exhibited slow oscillatory hyperpolarisations at a given concentration of caffeine (Table 1). The order of favourable anions was citrate > acetate and/or > sulphate > Cl.

There may be several interpretations for this order. First, assuming that both A- and C-hyperpolarisations are generated by the intracellular release and uptake cycle of Ca^{2+} at a Ca^{2+} storage site[11], this order of anions may indicate their ability to release Ca^{2+} from the storage site like caffeine; citrate would be most effective, and Cl^- would have no effect. The latter case would thus require caffeine. Second, the order of these anions may reflect depressant actions on the Ca^{2+} release mechanism in the ganglion cell. Endo[16] reported that a sustained treatment of the SR with a low concentration of Cl^- inactivated the release of Ca^{2+}. As the Ca^{2+} storage site in the ganglion cell seems to resemble the SR in its properties[11,13], Cl^- leaked out of an electrode into the cytoplasm may depress the Ca^{2+} release mechanism in the ganglion cell. This Cl^- action may be antagonised by caffeine. If so, citrate may be inert and A-hyperpolarisations could be a physiological phenomenon. We do not yet know which of these possibilities is operative. In any case, it is quite clear that the species of intracellular anions in the ganglion cell markedly affects the mechanism of the slow oscillatory hyperpolarisations.

It is unlikely that the present findings are the result of a chelating action of citrate because the estimated intracellular concentration of citrate was not high enough to produce a chelating action, and because such a chelating action would have blocked A-hyperpolarisations[11,13,14].

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Glucocorticosteroids modulate transmitter choice in developing superior cervical ganglion

I. S. McLennan, C. E. Hill & I. A. Hendry

Department of Pharmacology, John Curtin School of Medical Research,
Australian National University, PO Box 334, Canberra, ACT 2601,
Australia

***In vivo* the superior cervical ganglion (SCG) is adrenergic with less than 5% of neurones expressing cholinergic properties[1]. *In vitro* these neurones can develop either an adrenergic or cholinergic phenotype, depending on the nature of the culture conditions[2]: when immature SCG neurones are cultured in the virtual absence of non-neuronal cells they develop adrenergic properties, synthesising noradrenaline (NA) and a negligible amount of acetylcholine (ACh)[3,4]; when ganglionic non-neuronal cells are present the neurones develop a cholinergic phenotype, characterised by increased ACh synthesis and a corresponding decrease in NA synthesis[5]. If ganglionic non-neuronal cells release a factor which induces cholinergic properties *in vitro*, why is the SCG not cholinergic *in vivo*? *In vitro* there are none of the presynaptic, postsynaptic, or circulatory hormonal influences normally present in neuronal development. Depolarisation of SCG neurones *in vitro* prevents the development of cholinergic properties, suggesting that the preganglionic input *in vivo* may maintain the adrenergic nature of the SCG[6]. However, as prevention of depolarisation of neurones *in vivo* by preganglionic nerve section does not alter the adrenergic character of the SCG, influences other than the preganglionic input must be involved in preventing the development of cholinergic properties *in vivo*[7]. Circulatory glucocorticosteroids modulate the induction of neurotransmitter synthetic enzymes in the SCG[8,9] and have been reported to change the expression of adrenergic and cholinergic properties in a pheochromocytoma cell line[10]. We present here results suggesting that glucocorticosteroids are involved in specifying the transmitter type used in the SCG.**

SCG from 3-day-old rats were cultured on collagen-coated plastic multitrays (Linbro 76-033-05) in Dulbecco modified Eagle's medium containing 50 mM HEPES, 100 U ml^{-1} penicillin G, 10% inactivated horse serum, 1 μg ml^{-1} β-nerve growth factor (βNGF) and varying concentrations of dexamethasone (DXM) or corticosterone. After 14 days incubation choline acetyltransferase (CAT) and tyrosine hydroxylase (TH) activity were determined by previously described methods[12,13].

DXM antagonised the conversion of the SCG from an adrenergic to a cholinergic phenotype *in vitro*, resulting in a dose-related increase in the activity of TH and a corresponding decrease in CAT activity (Fig. 1*a*). The CAT:TH ratio, a sensitive measure of the degree of cholinergic character, decreased with increasing DXM concentration, reaching a minimum at 10^{-7} M DXM (Fig. 1*b*).

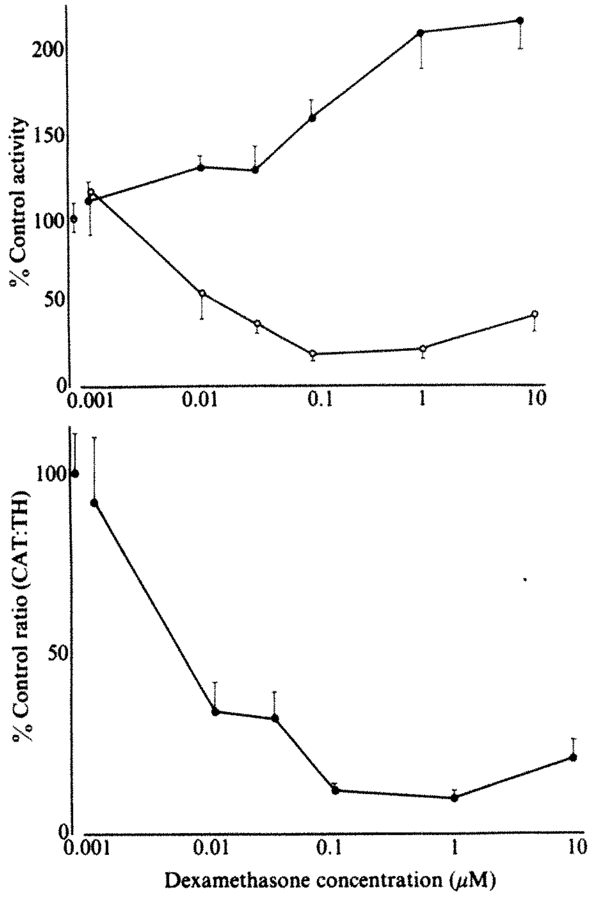

Fig. 1 Effect of dexamethasone on enzyme activities in cultured superior cervical ganglia. *a*, Effect on tyrosine hydroxylase (●) and choline acetyltransferase (○) levels; *b*, effect on the ratio of choline acetyltransferase to tyrosine hydroxylase activity. Activities are expressed as a percentage of the activity of control ganglia grown in the absence of dexamethasone. Control values were TH: 62 ± 5 pmol h^{-1} per ganglion; CAT: 0.76 ± 0.07 nmol h^{-1} per ganglion and CAT:TH 12.2 ± 1.3. Points are means ±s.e.m. for 12, 6, 6, 12, 12, 12, 12 ganglia respectively. All ganglia were cultured for 14 days and the medium was changed every 4 days.

Table 1 Effect of glucocorticoids on the ratio of choline acetyltransferase (CAT) to tyrosine hydroxylase (TH) in cultured rat superior cervical ganglia and comparison with the intrinsic ratio *in vivo*

	CAT:TH
In vitro	
Control	13.6 ± 1.8
Dexamethasone 10^{-7} M	1.0 ± 0.2
Corticosterone 10^{-6} M	0.5 ± 0.1
Corticosterone 10^{-5} M	0.0 ± 0.2
In vivo	
Decentralised	0.6 ± 0.1

Superior cervical ganglia from 3-day-old rat pups were decentralised or placed in culture and the activity of TH and CAT determined 14 days later. Ratios are means ±s.e.m. for six ganglia.

The naturally occurring glucocorticosteroid in rats is corticosterone. At the physiological plasma level for neonatal rats (1 μM)[14], this steroid was similar to DXM in its ability to alter the levels of CAT and TH. The ratio of CAT:TH in these cultures is comparable to the CAT:TH ratio observed in decentralised ganglia *in vivo* (Table 1).

These results indicate that physiological levels of glucocorticosteroids can influence the choice of transmitter type of the developing SCG *in vitro*. The glucocorticosteroids may be acting on (1) the non-neuronal cells to cause either a decrease in the production or liberation of a factor inducing cholinergic properties, (2) directly on the neurones to alter their responsiveness to such a factor or (3) selective destruction of cholinergic neurones. Irrespective of whether glucocorticosteroids act directly or indirectly on neurones it is likely that during the development of the SCG *in vivo* circulating levels of corticosterone are in part responsible for the adrenergic phenotype of the ganglion, despite the presence of non-neuronal cells which *in vitro* cause the SCG to become primarily cholinergic.

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Compact myelin exists in the absence of basic protein in the shiverer mutant mouse

D. A. Kirschner & A. L. Ganser

Department of Neuroscience, Children's Hospital Medical Center,
300 Longwood Avenue, Boston, Massachusetts 02115

Department of Neuropathology, Harvard Medical School, 25 Shattuck
Street, Boston, Massachusetts 02115

The myelin sheath is a multilamellar membrane system which surrounds axons in vertebrates and provides the electrical insulation necessary for saltatory nerve impulse conduction. Myelin forms from its cell of origin as a flattened, membrane-bound cytoplasmic process which wraps spirally around the axon; a periodic compact array of membrane pairs is produced from the wrappings as the cytoplasmic contents are extruded, and the external surfaces of membranes become apposed[1,2]. Neurological mutant mice which show myelin abnormalities are useful models for examining the formation, stability and breakdown of myelin. For example, the shiverer mouse carries an autosomal recessive mutation[3] (*shi*)[4] that results in severe myelin deficiency in the central nervous system (CNS)[5,6], apparently due to a defect in myelin formation[5,6]. The small amount of myelin that does form in the CNS is generally not compacted at its cytoplasmic surfaces[6], possibly due to the low level of basic protein in shiverer CNS tissue[7]. In the peripheral nervous system (PNS), in contrast, amounts of compact myelin seem to be normal[6]. The coarse tremor and convulsions that begin at about 2 weeks of age in the shiverer are presumably due to the severe CNS deficiency of myelin, as similar neurological signs are shown by other mutants with reduced CNS myelin[8]. Most studies on such mutants have concentrated on those regions of the nervous system which are grossly deficient in myelin[5–10]. In the other regions myelin seems by light microscopy to be normal. At the ultrastructural and molecular level, however, this myelin sometimes shows abnormalities[11–14], and this has prompted us to examine intensively such myelin in several neurological mutants. For this we have used X-ray diffraction, electron microscopy and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). We report here that, of the mutants we have examined so far, the shiverer mouse is unique in showing a striking alteration in myelin protein composition that does not significantly affect the gross morphology and lamellar organisation of the myelin sheath. Our results thus question the proposed role of basic proteins[15–19] in myelin as 'structural cement'.

X-ray diffraction patterns of myelin were obtained from freshly dissected sciatic nerves of adult normal and shiverer mice (Fig. 1*a*). The patterns extend to 0.06 Å^{-1} and are similar in their fundamental spacings and in the relative intensities of the reflections; this indicates that the packing and molecular organisation of the membranes in myelin from the shiverer mouse correspond closely to that from the normal mouse. The patterns also show comparable linewidths and total integrated intensities for the reflections, which means that the regularity of the packing of the membrane pairs in myelin and the total amount of myelin are similar in both animals. Small differences between the patterns, however, were consistently observed. Measurement of the normalised diffraction intensities shows that the first, fourth, sixth and eighth order reflections are increased and that the third and fifth orders are decreased in shiverer compared with the normal. These differences reveal an alteration in membrane bilayer structure of shiverer peripheral nerve myelin. Electron density profiles (Fig. 1*b*) calculated from the diffraction data show that in shiverer myelin there is a significant decrease in electron density on the cytoplasmic side of the bilayer both in the space between bilayers and at the position of the lipid polar head groups. Definitive assignment of these changes in electron density to particular molecular constituents requires chemical characterisation of shiverer peripheral nerve myelin.

Electron micrographs of adult shiverer mouse sciatic nerve show that the myelin is abundantly present as normally compact arrays of membrane pairs (Fig. 2). High magnification electron micrographs of shiverer myelin reveal typical major dense lines and double intraperiod lines which represent, respectively, the appositions of membranes at their cytoplasmic and at their extracellular surfaces. These results confirm the conclusions drawn from the X-ray diffraction measurements that the relative amounts of myelin and the regularity of membrane packing in normal and shiverer mice are similar. Moreover, electron micrographs of shiverer sciatic nerve do not reveal any obvious signs of myelin breakdown such as Schwann cell lysosomes filled with myelin debris or macrophage phagocytosis of the sheath.

The protein composition of myelin in normal and shiverer nerves was analysed with SDS-PAGE. Whole sciatic nerves were dissolved in SDS and electrophoresed in SDS on polyacrylamide gradient slab gels using the discontinuous buffer system of Maizel[20]. The myelin protein bands were identified by co-electrophoresing isolated myelin from normal mouse sciatic nerve and purified myelin proteins. Before examining the shiverer mutant, which is on a hybrid background in our laboratory, we compared the electrophoretic patterns of sciatic nerve proteins from six strains of normal mice (C57BL/6J, DBA/2J, BALB/CWt, C3H/HeJ, CBA/J and AKR/J). The patterns were virtually identical. Therefore, the hybrid background of shiverer would not be expected to produce any variations that might be seen in the banding patterns—variations would be attributable to the mutation.

The gel of normal and shiverer sciatic nerves from animals of different ages (Fig. 3) shows that the major myelin protein, P_0 glycoprotein[21], is present in comparable amounts at all ages. In contrast, the myelin basic proteins P_1 and P_2 (ref. 21) are present in normal but are absent from shiverer nerves. We also note small differences between shiverer and normal among certain minor protein bands of myelin. We are now examining whether the basic proteins are present transiently in shiverer before 2 weeks of age, during the earliest stages of myelin formation. We have also found that the basic proteins were undetected in adult shiverer sciatic nerve even after loading the gel with twice the usual amount. In trigeminal nerve of the shiverer mouse a similar absence of myelin basic proteins was found. The fact that no additional bands appear in the shiverer gels means that no other proteins are produced which could replace the basic proteins, with the possible exception of small peptides that could pass through the 18% acrylamide portion of the gel and be undetected.

The apparent lack of myelin basic proteins in peripheral nerves of the shiverer mouse might be explained by selective proteolytic activity during preparation for SDS-PAGE. We tested this possibility by three separate procedures. (1) Shiverer sciatic nerves were boiled for 2 min in 2% SDS immediately after dissection to denature proteases. (2) Shiverer sciatic nerves

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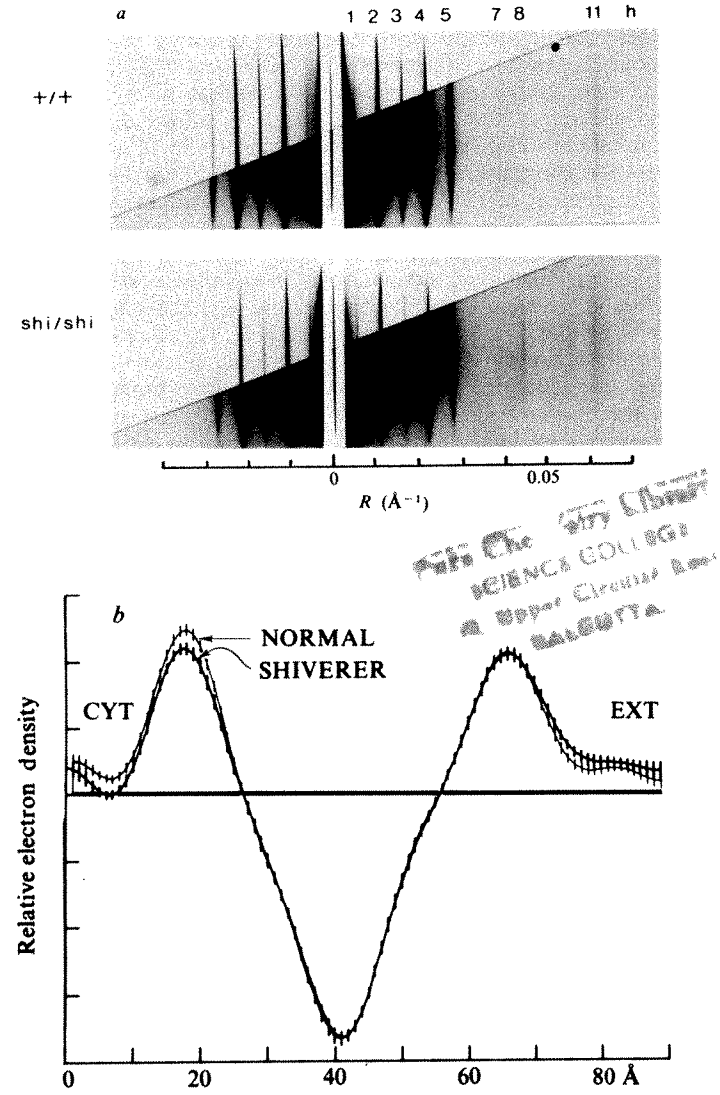

Fig. 1 X-ray diffraction from sciatic nerves of normal (+/+) and shiverer (*shi/shi*) mice. *a*, Diffraction patterns were obtained at room temperature from nerves mounted under tension in thin-walled quartz capillaries containing phosphate-buffered saline (0.9% NaCl, 0.01 M sodium phosphate, *p*H 7.4). The X-ray source was a fine-line Philips X-ray tube operated at 40 kV, 15 mA. Linear collimation and monochromatisation for CuKα radiation was achieved using a bent nickel-coated optical flat. The X-ray camera had a specimen-to-film distance of 200 mm. Exposure times were 12–20 h using Ilford Industrial G film. Top films and underfilms are presented to show both the strong, low order reflections and the weak, higher orders. The reciprocal space coordinate, R, is indicated along the bottom and the Bragg or diffraction orders, h, are numbered along the top. Both diffraction patterns show strong, even orders and weak, odd orders for $R < 0.03$ Å^{-1} and show weak, discrete reflections to $R \approx 0.06$ Å^{-1}. The most obvious differences between the patterns are the decreased intensities of the third and fifth orders, and the increased intensity of the first order in the shiverer. The spacings and optical densities of the diffraction orders were measured by a Joyce-Loebl MK III C microdensitometer. The repeat period measured for normal sciatic nerve myelin is 177 Å and for shiverer it is 178 Å; the 1-Å difference is less than the variability among different specimens. Measurements of the diffracted intensities were made on sciatic nerves from six normal and two shiverer mice. Integrated peak intensities, $I(h)$, were determined by planimetry after background subtraction using a Numonics digitiser interfaced to a PDP 11/05 computer. Structure amplitudes, $F(h)$, were calculated from $(hI(h))^{1/2}$ and were normalised so that $\sum_h F^2(h)/d = \text{constant}$[37]. The final sets of amplitudes that were used to derive the electron density profiles for the normal and shiverer myelin membranes were obtained by calculating a weighted average of the amplitudes for each diffraction order h. Individual standard deviations of the structure amplitudes were used as the weighting factors. These standard deviations arise from determining peak intensity over background, comparing the integrated intensity from corresponding peaks on either side of the direct beam, and scaling the weak and strong reflections recorded on different films during an exposure. The mean uncertainty of the measurements, calculated from the sum of the standard deviations of the amplitudes divided by the sum of the amplitudes, was about 6%. *b*, Electron density profiles of the myelin membranes were calculated from the structure amplitudes, $F(h)$, with phases assigned as previously described[12,38]. The profiles are plotted from the centres of the cytoplasmic fluid spaces (cyt) and extend to the centres of the extracellular fluid spaces (ext) between membrane bilayers; this represents half the membrane pair which constitutes the repeating unit of myelin. The short vertical bars on the density profiles show an uncertainty of 1 s.d. from the mean value of the profile[12]. The profiles were superposed to minimise the differences between the membrane bilayers. Overall, the membranes have similar electron density distributions. However, the shiverer myelin membrane does show a significant decrease in electron density at the polar head group region on the cytoplasmic half of the lipid bilayer. This could be accounted for by a loss of electron dense protein and/or of lipid polar groups. There is also in shiverer myelin a decrease of the average electron density in the cytoplasmic space which might be due to a local decrease in protein concentration.

were homogenised in 2% SDS containing three protease inhibitors (10^{-5} M leupeptin[22], 10^{-4} M *o*-phenanthroline[23], 10^{-3} M phenylmethyl sulphonyl fluoride[24]; Sigma). (3) The sciatic nerves of normal and shiverer mice were homogenised together in 2% SDS; in this mixture myelin basic proteins present in normal nerve would be exposed to any protease in shiverer nerve. Boiling or the use of protease inhibitors had no effect on the gel pattern of shiverer sciatic nerve. Mixing of shiverer nerve with the normal nerve did not alter the amount of basic proteins expected from the normal. We conclude that proteolytic activity does not explain the absence of basic proteins in shiverer peripheral nerve myelin.

Radioimmunoassay (RIA) provides a second, more sensitive method for detecting myelin basic proteins. A quantitative comparison of the levels of P_1 basic protein in normal and shiverer sciatic nerves was made (Table 1); inclusion of the detergent cetyltrimethylammonium bromide in the RIA buffer[25] ensured full expression of this protein. The value for the amount of P_1 basic protein in shiverer, using 1% nerve homogenates, is at the background level of the assay. This indicates at least a 200-fold reduction of basic protein in shiverer relative to normal. Determination of the absolute amount of P_1 basic protein in shiverer would depend on using more concentrated nerve homogenates. Failure to detect this protein in shiverer nerve might be due to inhibition of the antigen–antibody reaction in the RIA by some factor endogenous to shiverer nerves. We tested this possibility by carrying out the assay on a one-to-one mixture of normal and shiverer nerve homogenates. The level of P_1 basic protein measured was half the amount in normal nerve that would be expected if there were no interference with the antigen–antibody reaction. The RIA also indicates that immunoreactive fragments of P_1 basic protein are not present in shiverer nerve. We intend to quantitate the parallel lack of P_2 basic protein from shiverer peripheral nerves using RIA.

We have found that shiverer peripheral nerve myelin lacks basic proteins but that the lamellar arrangement of membranes—the repeat period and the widths of the cytoplasmic and extracellular spaces—are the same as in normal myelin. At the level of the individual myelin membrane bilayers, however, we find a small decrease in electron density of the polar head group region in the cytoplasmic half of the bilayer, which could be accounted for by the absence of basic proteins from that region. This decrease might also be explained by a reduced proportion of phosphate-containing lipids, a possibility that is now being examined.

Table 1 Radioimmunoassay of P_1 basic protein in mouse sciatic nerve

	µg P_1 per mg wet weight
Normal	4.05±0.46
Shiverer	0.019±0.015

1% (w/v) homogenates of sciatic nerves were made in RIA buffer containing 0.2 M Tris-acetate (*p*H 7.3), 1% cetyltrimethylammonium bromide, 1 mg ml^{-1} calf thymus histone, 0.5% (w/v) calf serum and 0.02% sodium azide. The homogenates were centrifuged to remove connective tissue and 10-µl aliquots of the supernatants, diluted 1 : 100 with RIA buffer for the normal and undiluted for the shiverer, were used for the assay. The values given represent the averages of assays on the sciatic nerves from each of three different normal and three different shiverer mice. The RIA was carried out as described by Cohen[25] and detects both A_1 and P_1 basic proteins.

The proposed role of basic proteins as 'structural cement' in myelin compaction and stabilisation[15-19] derives from the physicochemical properties of basic protein from the CNS, where it accounts for 25–50% of the total protein of isolated myelin[15]. Because it is an extrinsic membrane protein, that is, easily extracted by dilute acid or salt solutions[26], it is most probably localised to membrane surfaces. Large basic protein (named A_1 in CNS myelin) is highly cationic at physiological *p*H, with an isoelectric point of 10.5 (ref. 27) or 12–13 (ref. 28), and will complex with acidic lipids[29], with which it forms lamellar systems[30]. CNS basic protein is also able to interact hydrophobically with lipids[31], presumably due to the nonpolar regions in the protein molecule[32]. The basic proteins of PNS myelin have similar properties[15,30,31,33,34] and a similar structural role has therefore been suggested for them[17]. However, the lower amounts of basic proteins in PNS myelins, which range from 22% of total myelin protein in rabbit sciatic to as little as 6% in rat sciatic nerve[35], lead one to question such a structural role for basic proteins in the PNS. Moreover, the virtual absence of basic proteins from shiverer peripheral nerve myelin demonstrates that these proteins are not required to maintain stable, compact myelin in the PNS. Possibly, the function of structural cement in peripheral nerve myelin is provided by P_0 glycoprotein, which constitutes 50–65% of the total myelin protein[35]. In contrast, if CNS myelin does require basic protein as a structural component, the absence of this protein as a result of the shiverer mutation[7] would explain the defective myelin formation of the CNS[5,6]. A preliminary account of this work has been reported elsewhere[36].

We thank Professor R. L. Sidman for valuable discussions and encouragement, Dr J. Cowan and Mr M. Baker for providing the shiverer mice, Dr S. R. Cohen for carrying out the RIA,

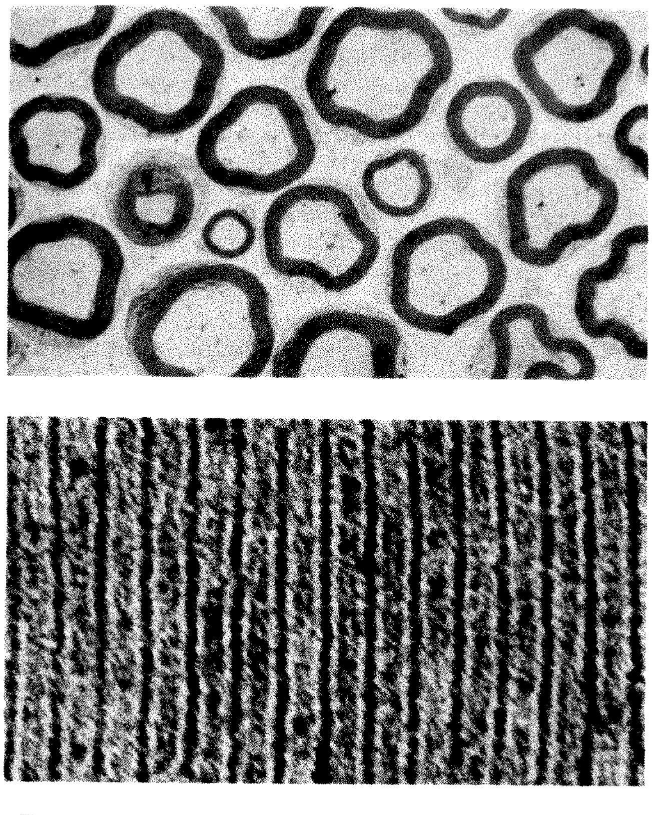

Fig. 2 Electron micrographs from shiverer sciatic nerve. Upper: low magnification view (×2,400) shows nerve fibres that seem to be normally myelinated. Lower: at high magnification (×434,000) the major dense lines and the double intraperiod lines of the array of myelin membranes are apparent. Continuity of the intraperiod lines can be seen more clearly by viewing the micrograph obliquely. Nerves were fixed with glutaraldehyde, postfixed with ferrocyanide-OsO_4, *en bloc* stained with uranyl acetate, dehydrated in methanol: methyl Cellosolve (1:1) and embedded in Epon; thin sections were counterstained with uranyl and lead.

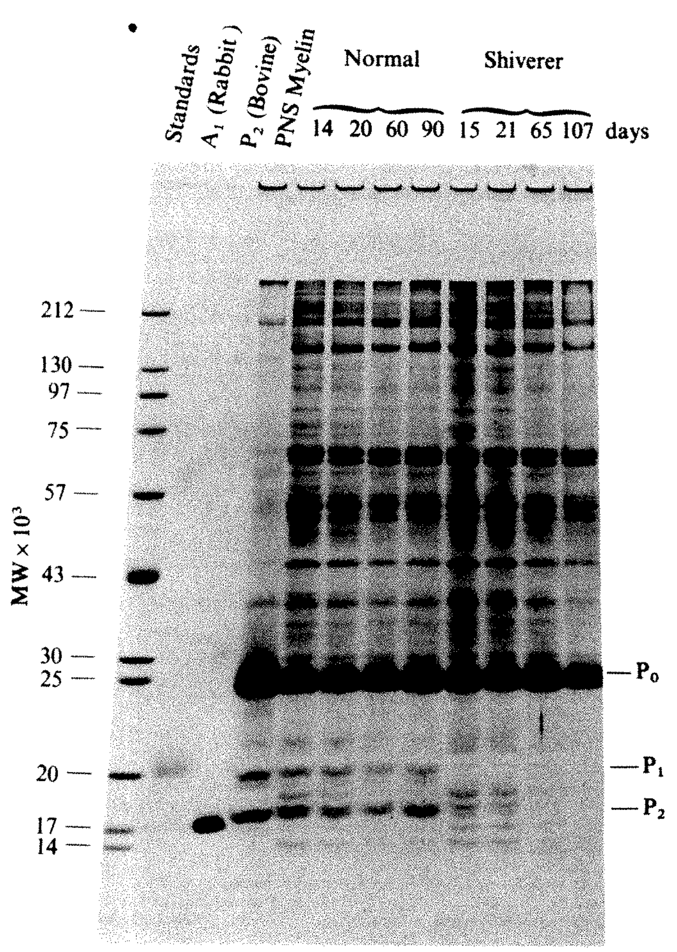

Fig. 3 SDS-PAGE analysis of protein composition of normal and shiverer sciatic nerves from animals of different ages. Nerve homogenates (15 mg wet weight per ml) were prepared in 2% SDS at room temperature, then diluted 1:1 with a mixture of 20 mM Tris-phosphate buffer at *p*H 6.7, 10% β-mercaptoethanol, 20% Ficoll and 0.002% bromphenol blue and finally stored frozen until electrophoresis. The electrophoretic system of Maizel[20] was used with the exceptions that the resolving gel was a slab 0.75 mm thick and 12 cm long, with an 8–18% gradient of acrylamide, and the stacking gel was 4% and made up in 62.5 mM Tris-phosphate at *p*H 6.7. Sample aliquots of 20 μl per lane were electrophoresed at room temperature at a constant voltage of 40 V for 2 h, which brought the dye front into the resolving gel, and then 180 V for 4.5 h. The gels were fixed overnight in 50% methanol–10% acetic acid and subsequently stained for 1 h with 0.1% Coomassie Brilliant Blue R in 50% methanol–10% acetic acid. After destaining in 10% methanol–10% acetic acid the gels were photographed through a yellow filter (Kodak K 2). The molecular weight (MW) standards are: myosin at 212,000, β-galactosidase at 130,000, phosphorylase *a* at 97,000, conalbumin at 75,000, pyruvate kinase at 57,000, ovalbumin at 43,000, carbonic anhydrase at 30,000, chymotrypsinogen A at 25,000, soybean trypsin inhibitor at 20,000, myoglobin at 17,000, and lysozyme at 14,000. Myelin basic protein A_1, prepared from rabbit brain[39], is quite similar to P_1 basic protein of PNS[33]. Myelin basic protein P_2 was prepared from bovine intradural spinal roots[40]. PNS myelin was purified[41] from sciatic nerves of normal C57BL/6J mice. The major myelin proteins[21] of the sciatic nerve homogenates, P_0 glycoprotein and P_1 and P_2 basic proteins, are identified by comparison with the electrophoretic banding patterns of purified myelin basic proteins from other species and of myelin isolated from mouse sciatic nerves. P_1 and P_2 migrate in this gel system at the apparent MWs of ~20,000 and ~18,000; the actual MWs calculated from amino acid analysis of rabbit P_1 is 18,000 (ref. 21) and of bovine P_2 is 14,300 (ref. 42). Of the myelin proteins, P_0 glycoprotein is prominent in the gels of normal and shiverer mice, whereas P_1 and P_2 basic proteins are not visible in the shiverer. Two- and three-week-old normal and shiverer mice show more bands in the low MW region of the gels than do the adults. Two of these bands border the P_2 protein and are more apparent in shiverer because of the absence of this basic protein. They are, however, present in gels of normal nerves, especially at 14 days. Apart from the myelin proteins, the banding patterns at corresponding ages are virtually identical.

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Evolution and gene transfer in purple photosynthetic bacteria

Richard E. Dickerson

Norman W. Church Laboratory of Chemical Biology, California Institute of Technology, Pasadena, California 91125

A concern voiced in connection with recent sequencing of cytochrome *c* from the Rhodospirillaceae or purple non-sulphur photosynthetic bacteria[1-3] is that molecular information might be of little use in deciphering bacterial phylogeny because of the possibility of lateral transfer of genes and the consequent scrambling of the genetic record. This could be true for many proteins, of course, but the immediate question is, is it true for cytochromes *c*, for which extensive primary sequence[1-3] and X-ray structure[4-10] information is available for comparison? The evidence suggests that this is probably not so. The disagreement between cytochrome *c* sequences and the standard taxonomy of the Rhodospirillaceae in Bergey's Manual[11] is not a problem. That reference, as its title indicates, is a manual of determinative rather than evolutionary bacteriology. Its goal is a reproducible system for identification of bacteria. If one claims that these determinative categories also have phylogenetic or evolutionary meaning, this is an assertion that must be proven. The argument outlined below suggests that molecular traits may eventually become more a dependable basis for classification of the Rhodospirillaceae than is gross morphology.

Table 1 contains information rearranged from Table 1 of Ambler *et al.*[1], with the addition of two more species, and with further information about the positions of chain insertions in the cytochromes *c* and sequence comparisons between the 16S RNA subunits of bacterial ribosomes. The ribosomal RNA classification is discussed in greater detail in the following paper[12], and more detailed information on cell shape and infolding of the photosynthetic membrane may be found in Pfennig and Trüper[13]. X-ray crystal structure analyses reveal that the cytochromes c_2 and c_{551} from the Rhodospirillaceae fall naturally into three structural classes, each of which has a parallel among purely respiratory cytochromes c_{551}, *c* or c_{550}. These structural classes are shown in Fig. 1. The short (or S) cytochromes c_{551} from *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue* resemble, in both sequence and folding, the respiratory c_{551} from pseudomonads and *Azotobacter* (Fig. 1*a*). Cytochromes *c* of the medium or 'mitochondrial' (M) class are similar in length and sequence to cytochrome *c* from eukaryotic mitochondria, and all have the extra chain at the bottom of the molecule that is shaded and marked by I in Fig. 1*b*. Others, the long or L class, resemble *Paracoccus* c_{550} by having the shaded insertions marked II and III in Fig. 1*c*, and sometimes insertion IV as well. Moreover, the inserted amino acids in these photosynthetic and respiratory cytochromes *c* are similar in sequence, arguing for a common evolutionary origin. All these molecules—*c*, c_2, c_{550}, c_{551} and c_{555} (from *Chlorobium*)—seem to make up one large evolutionarily related family of proteins. The presence or absence of chain insertions I, II, III and IV is listed as one of the traits in Table 1.

In Table 2, the amino acid sequences of cytochrome c_2 or c_{551} from the Rhodospirillaceae are compared quantitatively in two different ways. Below the matrix diagonal, which contains the total number of amino acids in each protein, are tabulated the number of identical positions in pairwise comparisons of sequences. The number of differences between sequences is found above the diagonal. (As is customary, a deletion in one chain but not the other is included in the tally of differences.) For closely related sequences, the difference matrix is best for showing changes in sequence, but for more distantly related sequences such as these, the identities matrix is better. It avoids the difficult question of whether to treat a long chain deletion as one evolutionary event or many, and provides a measure of how nearly identical two sequences are in those regions of chain that they have in common. Gross insertions or deletions in the chain (such as regions I–IV) can then be assessed separately.

If the information in Tables 1 and 2 is arranged by Bergey genera, the principal impression is one of confusion. However, if the Bergey genera are set aside, a strong correlation can be seen between the size and sequence of cytochromes *c*, type of infolding of outer bacterial membrane, and 16S ribosomal RNA sequences. Those species that have class II RNA sequences also have short cytochromes *c* and tubular membrane infoldings, even though they fall into three different Bergey genera. (The amino acid sequence of *Rhodocyclus purpureus* cytochrome is not known, but one would predict that it is a short cytochrome.) Both the identity and the difference data in Table 2 show that the S cytochromes *c* are very different in sequences from the M and L (a mean and mean deviation of 16.4 ± 2.2 identities between S and M or L). The M and L classes are more nearly alike, but there are fewer identities between M and L (38.6 ± 3.3 identities) than between different M (50.3 ± 8.3) or L (49.4 ± 6.1). The presence or absence of insertions II, III and IV, of course, draws a clear distinction between M and L cytochromes.

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Table 1 Comparison of properties of purple photobacteria and their cytochromes c_2 or c_{551}

	RNA Class	Cytochrome size	Chain insertions (Fig. 1) I	II	III	● IV	Type of photosynthetic membrane	Mode of cell division	Cell form
Chromatiaceae	III	?	?	?	?	?	Mostly vesicular	Fission	Various
Rhodospirillaceae									
Rps. gelatinosa	II	S	No	No	No	No	Tubular	Fission	Small rod
R. tenue	II	S	No	No	No	No	Tubular	Fission	Thin spiral
Rcy. purpureus	II	?	?	?	?	?	Tubular	Fission	Arc or circle
*R. fulvum**		M	Yes	No	No	No	Lamellar	Fission	Short spiral
*R. molischianum**		M	Yes	No	No	No	Lamellar	Fission	Spiral
Rps. globiformis		M	Yes	No	No	No	Vesicular	Fission	Sphere
Rm. vannielii	I	M	Yes	No	No	No	Lamellar	Budding	Stalked ovoid
Rps. viridis	I	M	Yes	No	No	No	Lamellar	Budding	Rod
Rps. acidophila		M	Yes	No	No	No	Lamellar	Budding	Large rod
Rps. palustris	I	L	Yes	Yes	Extra	No	Lamellar	Budding	Rod
R. photometricum		L	Yes	Yes	Yes	No	Lamellar	Fission	Large spiral
R. rubrum	I	L	Yes	Yes	Yes	No	Vesicular	Fission	Spiral
Rps. capsulata	I	L	Yes	Yes	Yes	Yes	Vesicular	Fission	Rod
Rps. sphaeroides	I	L	Yes	Yes	Yes	Yes	Vesicular	Fission	Sphere

Abbreviations: *R.*, *Rhodospirillum*; *Rps.*, *Rhodopseudomonas*; *Rm.*, *Rhodomicrobium*; *Rcy.*, *Rhodocyclus*. S, M, L: short, medium and long cytochromes *c* as described in the text.

* Possess.two similar isozymes of c_2.

Rps. gelatinosa, *R. tenue* and *Rcy. purpureus* show a diversity of cell shapes, which led to their assignment to different genera in the original Bergey classification. If one accepts this separation, and argues that these three species show common cytochrome properties because of lateral gene transfer, one must not only assume that the genes for cytochromes *c* and for photosynthetic membrane configuration are closely linked and jointly transferrable, but that they are both also linked to the genes responsible for the 16S RNA components of their ribosomes. At this point, gross morphology begins to look like the weakest of the available traits for phylogenetic classification.

Those species with M or L cytochromes *c* all have class I ribosomal RNA sequences. Their cell membrane infoldings are vesicles or stacked lamellae, never tubules. The cells reproduce either by simple binary fission, or in four species by asymmetric budding. The limited information in Tables 1 and 2 does not provide a clear basis for subgroupings. One should keep an open mind on the subject until more information is available, perhaps from sequences and structures of proteins that are quite different from the cytochromes. However, Table 2 does indicate close affinities between ***Rhodopseudomonas sphaeroides*** and ***Rhodopseudomonas capsulata***, between ***Rhodospirillum rubrum***

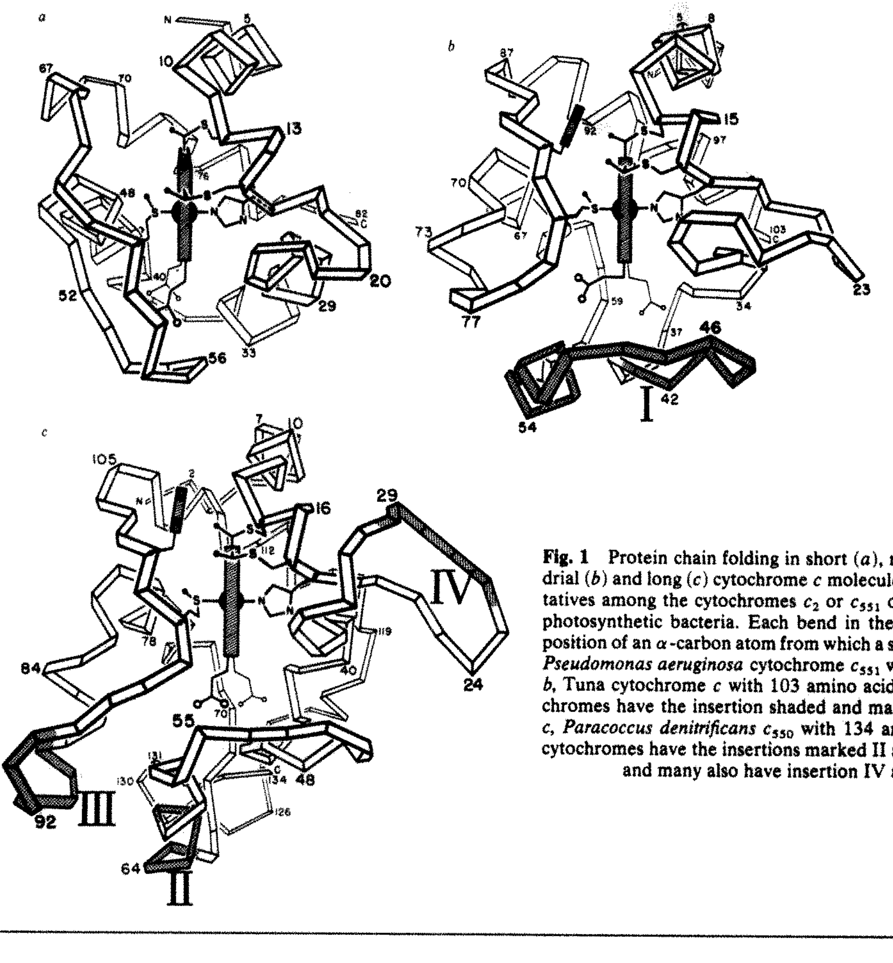

Fig. 1 Protein chain folding in short (*a*), medium or mitochondrial (*b*) and long (*c*) cytochrome *c* molecules. All have representatives among the cytochromes c_2 or c_{551} of purple non-sulphur photosynthetic bacteria. Each bend in the ribbon indicates the position of an α-carbon atom from which a side chain branches. *a*, *Pseudomonas aeruginosa* cytochrome c_{551} with 82 amino acids[10]. *b*, Tuna cytochrome *c* with 103 amino acids[8]. All medium cytochromes have the insertion shaded and marked I at the bottom. *c*, *Paracoccus denitrificans* c_{550} with 134 amino acids[6]. All long cytochromes have the insertions marked II and III at the bottom, and many also have insertion IV at the right.

Table 2 Pairwise sequence comparisons of cytochromes *c*

Italic numbers on the diagonal give the length of each chain. Below diagonal: total number of identical positions. Above diagonal: total number of differences. Sequences lettered at top as at far left.

	Cytochrome size	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A *Rps gelatinosa*	S	*85*	65	—	89	89	100	96	98	96	108	104	102	109	117
B *R. tenue*	S	30	*92*	—	99	101	103	100	102	97	105	99	95	107	114
C *Rcy. purpureus*	?	—	—	—	—	—	—	—	—	—	—	—	—	—	—
D *R. fulvum**	M	18	16	—	*99*	12	71	58	65	66	82	77	77	85	94
E *R. molischianum**	M	18	14	—	88	*100*	68	56	66	64	80	74	78	86	94
F *Rps. globiformis*	M	12	16	—	37	40	*106*	62	66	65	83	81	82	92	97
G *Rm. vannielii*	M	15	16	—	48	50	47	*104*	52	48	73	76	74	85	89
H *Rps. viridis*	M	16	14	—	44	43	47	56	*107*	47	76	79	79	84	91
I *Rps. acidophila*	M	18	19	—	43	45	47	59	61	*107*	80	80	76	90	81
J *Rps. palustris*	L	13	18	—	37	39	40	48	48	44	*114*	74	72	72	88
K *R. photometricum*	L	14	21	—	39	42	37	40	41	39	45	*113*	50	79	82
L *R. rubrum*	L	16	25	—	38	37	36	41	40	42	46	63	*112*	68	75
M *Rps. capsulata*	L	15	18	—	36	35	33	36	40	34	52	41	51	*116*	62
N *Rps. sphaeroides*	L	13	15	—	33	33	32	36	37	46	42	43	49	62	*124*

* Iso-2 is similar.

and *Rhodospirillum photometricum*, between three and possibly the fourth budding species, and between *Rhodospirillum molischianum* and *Rhodospirillum fulvum*, all of which are reasonable on other grounds. We have very tentatively drawn the phylogenetic tree shown in Fig. 2 as the basis for future discussion of new data. It suggests a point that we believe to be quite important, and which was first enunciated by Broda[14,15]: the polyphyletic origin of respiring bacteria from more than one line of photosynthetic ancestors.

It may be that lateral gene transfer and blurring of the evolutionary record will be a serious problem for some proteins, especially those that are intrinsically useful to a bacterium in their own right, without an attendant metabolic setting. However, with the proper choice of proteins this difficulty should be capable of being overcome, and the cytochrome *c* record provides grounds for optimism.

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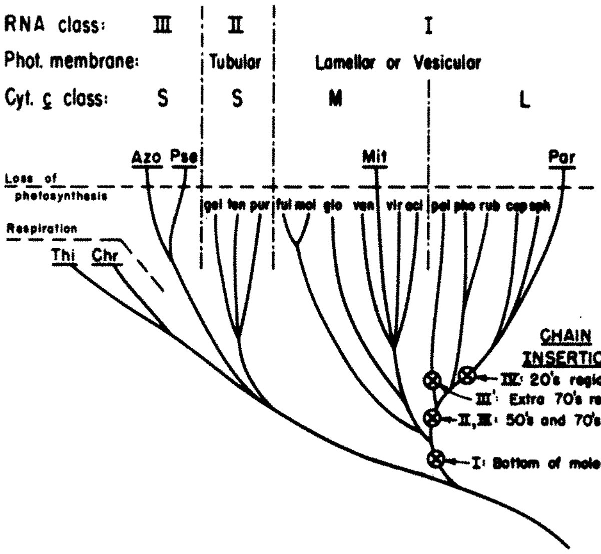

Fig. 2 Tentative phylogenetic tree of the purple photobacteria, based on the metabolic, morphological, protein and RNA data presented in Tables 1 and 2. The original cytochrome *c* is assumed to have been small, like *Chlorobium* c_{555} and *Anacystis* c_{554}. Circled crosses mark the four places where specific insertions of polypeptide chain are presumed to have occurred. Azo, *Azotobacter*; Pse, *Pseudomonas*; Mit, eukaryotic mitochondria; Par, *Paracoccus denitrificans*; Thi, *Thiocapsa*; Chr, *Chromatium*; gel, *Rps. gelatinosa*; ten, *R. tenue*; pur, *Rhodocyclus purpureus*; ful, *R. fulvum*; mol, *R. molischianum*; glo, *Rps. globiformis*; van, *Rm. vannielii*; vir, *Rps. viridis*; aci, *Rps. acidophila*; pal, *Rps. palustris*; pho, *R. photometricum*; rub, *R. rubrum*; cap, *Rps. capsulata*; sph, *Rps. sphaeroides*.

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Do genealogical patterns in purple photosynthetic bacteria reflect interspecific gene transfer?

Carl R. Woese*, Jane Gibson† & George E. Fox‡

* Department of Genetics and Development, University of Illinois, Urbana, Illinois 61801
† Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853
‡ Department of Biophysical Sciences, University of Houston, Houston, Texas 77004

It is generally thought that interspecific (lateral) transfer of genes is so extensive among bacteria that it is difficult, and perhaps impossible, to determine their phylogenetic relationships. Ambler and coworkers[1] reflect this in their suggestion that the relationships seen among cytochrome *c* sequences of the Rhodospirillaceae are merely the result of a haphazard lateral transfer of the particular gene, and give no indication of the true bacterial phylogenies. However, if comparative analysis of several unrelated macromolecules yields essentially the same phylogenetic tree, then that pattern is extremely unlikely to reflect the lateral transfer of genes[2]. We have also determined 16S ribosomal RNA catalogues[3] for many of the Rhodospirillaceae investigated by Ambler *et al.* and here we use these two sets of data to compare molecular phylogenies for these bacteria.

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The conclusion of Ambler *et al.*[1], suggesting that the relationships seen among cytochrome *c* sequences of the Rhodospirillaceae are merely due to random lateral transfer of the relevant gene, is based on three arguments. First, within the purple photosynthetic bacteria there is 'no correlation between a species' cytochrome *c* amino acid sequence and its phylogenetic position'; second, the cytochromes *c* from some of the Rhodospirillaceae seem to be more closely related to those from certain nonphotosynthetic organisms, for example pseudomonads, than they are to those from other members of the Rhodospirillaceae; and third, three different proteins—cytochrome c_{551}, cytochrome c', and the high potential iron sulphur protein—seem to produce three dissimilar phylogenetic trees for the same (small) set of organisms[1,4]. The first two arguments assume that we already know the true phylogeny for the Rhodospirillaceae, which is not the case. The genera now defined for the purple photosynthetic bacteria are not intended to be phylogenetic categories[5,6]; generally agreed phylogenetic categories for these organisms have never been proposed. In addition, the second argument seems to reflect the idea that the photosynthetic bacteria must be phylogenetically quite distinct from nonphotosynthetic bacteria (with perhaps a few exceptions). Although this view is widely accepted[7,8], it has no factual support.

Our objection to the lateral transfer explanation is not based primarily on counter-argument, however, but on the facts available. If a phylogeny derived from a comparative analysis of one macromolecular species reflects lateral transfer of the underlying gene, then it is virtually impossible that the same phylogeny will result when another functionally unrelated macromolecule is used as the phylogenetic probe[2].

Many of the Rhodospirillaceae whose cytochromes *c* have been sequenced by Ambler *et al.*[1] have also been characterised in terms of their 16S ribosomal RNAs using the oligonucleotide cataloguing method[3]. Both data sets are analysed by comparable methods: the 16S rRNA catalogues are compared by the binary association coefficient S_{AB} as described previously[9]; and the cytochrome *c* data are represented as per cent sequence homology for each pair of sequences (using the alignments of Dickerson[10], see Table 2 of the accompanying paper[10]). Cluster analysis (average linkage between the merged groups) on these two data sets generates the dendrograms shown in Fig. 1.

The two patterns are in excellent agreement. It is therefore highly unlikely that either one reflects the lateral transfer of genes. Moreover, a number of phenotypic properties of these organisms are consistent with the groups so defined (for details see refs 3, 6 and 10).

The 16S rRNA data indicate that the purple photosynthetic bacteria fall into three major classes—that defined by the bulk of the *Rhodopseudomonas* species and their relatives (class I), that defined by *Rhodopseudomonas gelatinosa* and *Rhodospirillum tenue* (class II) and that defined by *Chromatium* (class III). The first two classes, which comprise the purple non-sulphur bacteria, give no evidence of being specifically related to one another to the exclusion of the last class, the purple sulphur bacteria. Thus if, as has been suggested[10], the former phenotype evolved from the latter, it may have done so several times.

Although a comprehensive survey of 16S rRNA catalogues for the Gram-negative bacteria is not yet available, it is already apparent that the genealogical cluster defined by the purple photosynthetic bacteria is far more extensive phylogenetically than had been generally realised; each of the above-defined three classes of purple photosynthetic bacteria also contains representatives of various major nonphotosynthetic Gram-negative groups, for example, pseudomonads, enterics and vibrios[8]. Thus it would seem that many of the Gram-negative bacteria have arisen as nonphotosynthetic offshoots of photosynthetic ancestors, and the theory that the photosynthetic groups of bacteria are largely phylogenetically distinct from nonphotosynthetic groups is incorrect.

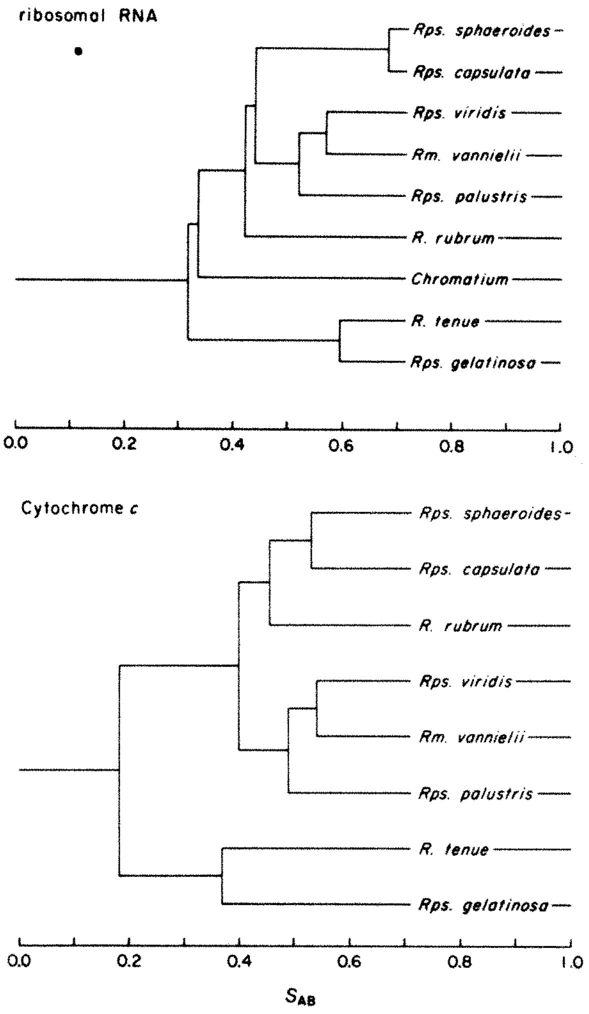

Fig. 1 The relationship among various purple photosynthetic bacteria as determined by 16S ribosomal RNA sequence comparisons, and cytochrome *c* sequence comparisons. *Rps.*, *Rhodopseudomonas*, *R.*, *Rhodospirillum*; *Rm.*, *Rhodomicrobium*.

An alternative explanation for the third argument of Ambler *et al.*—the non-congruence of phylogenetic trees derived from different proteins—is that their finding reflects the limitations in the use of small macromolecules such as cytochrome *c* for genealogical measurement. For a macromolecule to be useful as an evolutionary clock it must maintain functional and tertiary structural constancy over the group of organisms being considered[11,12]. Changing these collective properties seemingly involves clusters of selected, and therefore relatively rapid, changes in genetic sequence, making the molecule an inexact measure of phylogenetic distance at best. Bacterial genealogies encompass considerably greater phylogenetic distances and varieties of niches than genealogies within the higher organisms. Consequently it is not surprising that the conditions of constancy of tertiary structure and function are often not found in the bacteria. The three types of protein studies by Ambler *et al.*—cytochromes c_2 and c_{551}, cytochrome c' and the high potential iron sulphur protein[4]—seem to confirm this point. It is, therefore, not surprising that these three types of macromolecules yield non-congruent phylogenetic trees. For reasons not discussed here we feel that a large macromolecule such as the 16S rRNA is less susceptible to the difficulties which tend to arise when using the smaller macromolecules as genealogical probes.

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Isolation of the structural gene for alcohol dehydrogenase by genetic complementation in yeast

Valerie M. Williamson, Jeffrey Bennetzen & Elton T. Young

Department of Biochemistry

Kim Nasmyth & Benjamin D. Hall

Department of Genetics, University of Washington, Seattle, Washington 98195

Alcohol dehydrogenase (ADH) catalyses the interconversion of acetaldehyde and ethanol. This reaction is the final step during alcoholic fermentation in yeast and the initial step in the metabolism of ethanol in a wide variety of organisms. The regulation of ADH is now being studied in several organisms, notably yeast[1–4], *Drosophila*[5], maize[6] and man[7,8]. These organisms contain multiple ADH isozymes which are differentially expressed. The budding yeast *Saccharomyces cerevisiae* is particularly suitable for study because of the potential for genetic analysis of enzyme regulation and structure. Yeast possesses three distinct ADH isozymes[2]. In the presence of glucose ADHI (the 'classical' yeast ADH) is expressed whereas ADHII is repressed. A third enzyme, mADH, is associated with mitochondria. Mutants lacking each of these enzymes have been isolated by virtue of their greater tolerance to allyl alcohol[1], which is converted by ADH to the toxin, acrolein. There is no evidence as to whether the regulation of any one of these enzymes is under transcriptional control. Availability of recombinant DNA clones containing the respective genes would be of obvious value in resolving this problem. We describe here the use of the newly developed technique of yeast transformation[9–11] to isolate recombinant plasmids that contain the structural gene for ADHI (*ADC*1). These plasmids were selected from a pool of plasmids containing yeast DNA by virtue of their ability to complement the growth defect of yeast strains completely lacking ADH activity. Using DNA sequencing, they have been shown to contain the structural gene for ADHI.

The vector used for constructing the yeast DNA pool was the plasmid YRp7 (ref. 11). YRp7 is composed of a 1.4-kilobase *Eco*RI fragment containing the yeast *TRP*1 gene inserted into the *Eco*RI site of pBR322 (ref. 12). The fragment containing *TRP*1 confers on the hybrid plasmid the ability to replicate autonomously in yeast as a low copy number closed circular molecule[11]. Because it need not integrate into the chromosome, YRp7 will transform *trp*1 strains with high efficiency (10^4–10^5 transformants per μg DNA).

The construction of a pool of yeast sequences in YRp7 will be described in more detail elsewhere (K. N. and S. I. Reed, in preparation). Briefly, yeast DNA was partially cleaved with restriction endonuclease *Sau*3A, a 4-base pair recognition site enzyme which produces cohesive ends homologous to those produced by BamHI. The DNA fragments were separated by centrifugation in sucrose gradients and fragments larger than 5 kilobases were ligated into the *Bam*HI site in the tetracycline-resistance gene of YRp7. The pool of recombinant plasmids so created (>25,000 independent tet^s clones) was stored and amplified by transformation of *Escherichia coli* to ampicillin resistance.

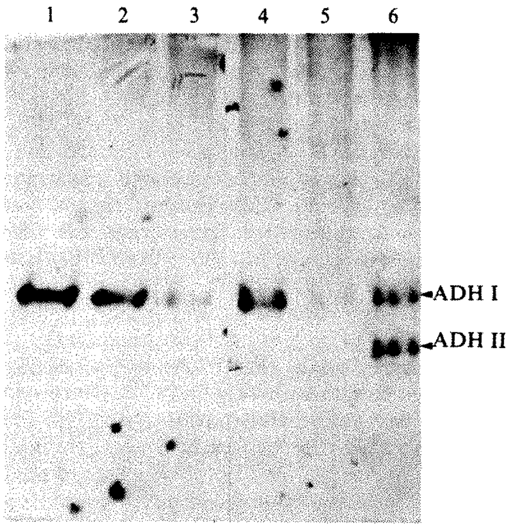

Fig. 1 Screening of transformed yeast for ADH activity. A *trp*1 strain lacking all ADH isozymes (302-21; *mat*α, *trp*1, *his*4, *adc*1, *adm*, *adr*2) was selected from a cross of strain SF-1C-M3 (donated by M. Ciriacy) and strain H293-4-2 (provided by L. Hartwell). The procedure for yeast transformation was essentially that described by Beggs[10]. Cells were plated in a 3% agar overlay lacking tryptophan and containing 2% glucose. The nine large Trp^+ colonies obtained after transformation with the YRp7 yeast pool were plated on medium lacking tryptophan. Extracts from cells grown to stationary phase in medium lacking tryptophan were analysed for ADH activity by spectrophotometric analysis of NAD^+ reduction when ethanol was added as substrate[1]. Aliquots of cell extracts containing approximately 22 μg of protein were electrophoresed into a 5% polyacrylamide slab gel. The electrophoresis system used was adapted from the Tris–glycine system of Davis[18]. The sample was applied to the gel in buffer containing 50 mM Tris–HCl, *p*H 6.8, 25% glycerol and 100 mM β-mercaptoethanol, with bromphenol blue as an indicator. The stacking gel consisted of 3% acrylamide, 0.08% bisacrylamide, 0.12% TEMED, 0.2% ammonium persulphate and 125 mM Tris–HCl, *p*H 6.8. The running gel consisted of 5% acrylamide, 0.13% bisacrylamide, 0.03% TEMED, 0.3% ammonium persulphate and 375 mM Tris–HCl, *p*H 8.8. Electrophoresis buffer was 25 mM Tris and 192 mM glycine and electrophoresis was carried out at 150 V for 3 h at 4 °C. The gel was then stained for ADH activity[19]. In the example shown, wells 1–5 contain extracts from the transformants 9T1–9T5, respectively. Well 6 contains extract from the strain which was used as the yeast DNA source in constructing the DNA vector pool.

The selective technique used for identifying plasmids containing an ADH gene was based on the inability of yeast mutants lacking ADHI to grow in the absence of respiration[13]. Such mutants will not grow at all in the presence of antimycin A (M. Ciriacy, personal communication) and grow only poorly when embedded in a 3% agar overlay. A *trp*1 strain lacking all ADH isozymes was transformed with plasmid DNA prepared from the pool using the technique described by Beggs[10] (see Fig. 1 legend). Nine large colonies (9T1–9T6; 10T1–10T3), a frequency of one per 4×10^3 Trp^+ transformants, were produced after incubation at 30 °C for 1 week. Seven out of the nine contained ADH activity after growth to stationary phase (Fig. 1). When analysed by electrophoresis on a native polyacrylamide gel, all seven displayed a band of activity co-migrating with ADHI from the donor strain (Fig. 1).

The Adc^+ transformants fell into two classes. Two, 9T2 and 10T2, had ADHI levels comparable to wild type. They were allyl alcohol sensitive, antimycin A resistant and stable for the Trp^+ phenotype. These stable transformants were further analysed by mating with an ADH-null *trp*1 strain. Tetrad analysis of the haploid progeny showed that both the Trp^+ and

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Adc$^+$ phenotypes segregated 2:2 (Trp$^+$Adc$^+$:Trp$^-$Adc$^-$), that is, only parental combinations were observed. This result indicates that these normally unlinked genes are now linked and have integrated into a chromosomal site. The site of integration was not determined. The second class of transformants contained 10–100% of the wild-type ADHI activity. When plated on YEPD plates, cells from these transformants produced a mixture of large and small colonies and rapidly lost the ability to grow without tryptophan. These colonies presumably contained autonomously replicating plasmids which were readily lost in the absence of selection, a property characteristic of YRp7 (ref. 11).

Plasmid DNA was isolated from transformant 9T6 and amplified in *E. coli* as described in Fig. 2 legend. Restriction enzyme digestion of the plasmid DNA (pY9T6) isolated from three different *E. coli* clones transformed with plasmid DNA from transformant 9T6 showed that all were identical. Three different plasmids, one of which (pY9T5) shared common yeast restriction fragments with pY9T6, were similarly isolated from yeast transformant 9T5.

Plasmids pY9T5 and pY9T6 were used to retransform strain 302–21 (ADH-null). With pY9T6, 0.06% of the cells surviving treatment were transformed to Trp$^+$, and with pY9T5, 0.02% were similarly transformed. All Trp+ cells were able to grow in the presence of antimycin A. Eight transformants were grown in medium lacking tryptophan and the extracts were assayed for ADH activity. All had near wild-type levels of ADH activity and produced an active enzyme which co-migrated with ADHI during electrophoresis. Both the Trp$^+$ and Adc$^+$ phenotypes were unstable in the absence of selection and were lost coordinately.

The above results clearly show that both pY9T5 and pY9T6 are capable of complementing the ADHI deficiency of 302–21 caused by the *adc*1 mutation. However, apart from the fact that ADHI-defective mutants have been found only at this locus[2], there is little compelling evidence that the *ADC*1 locus in fact constitutes the structural gene for the enzyme. To resolve this problem, we have tested the ability of pY9T5 and pY9T6 to hyridise to a ^{32}p-labelled cDNA probe prepared from an RNA fraction highly enriched for ADHI mRNA (J.B., unpublished). Both plasmids hybridised strongly (results not shown), suggesting that the locus is indeed the structural gene. Restriction maps of pY9T5 and pY9T6 (Fig. 2) show that the two plasmids contain about 4.1 kilobases of overlapping sequence but that their inserts are of different sizes and are in the opposite orientation in the YRp7 vector. DNA sequence was obtained

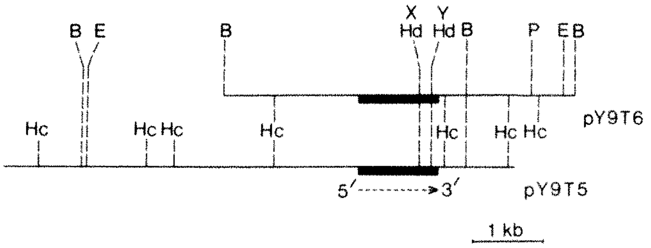

Fig. 2 Restriction endonuclease cleavage sites on the overlapping yeast DNA inserts of recombinant plasmids pY9T5 and pY9T6. Plasmid DNA was prepared as described by Livingston and Klein[20] from the unstable yeast transformants after growth in the absence of tryptophan and in the presence of antimycin A. Transformation of *E. coli* to ampicillin resistance produced approximately 100 colonies per µg DNA, all of which were tetracycline sensitive. Plasmid DNA was purified from *E. coli* by a modification of the procedure of Elwell *et al.*[21]. Single and double restriction digests of the DNA were analysed on horizontal 0.7% agarose gels. The position of the *ADC*1 locus was first determined by hybridisation to Southern filter[22] replicas of these agarose gels with radioactive cDNA complementary to an ADHI mRNA-enriched RNA fraction. A detailed description of the preparation and use of this cDNA will be presented elsewhere. The position and extent of the *ADC*1 structural locus was further defined by the DNA sequencing results (Fig. 3). The cleavage sites of relevant restriction nucleases are designated as follows: B(*BamHI*), E(*Eco*RI), Hc(*Hinc*II), Hd (*Hin*dIII), P(*Pst*I); X and Y designate specific *Hin*dIII sites which are referred to in the text and Fig. 3. The extent and position of the structural *ADC*1 locus is shown by the solid bars and the direction of transcription by the arrow below the bars. Restriction maps of the entire plasmids (not shown) demonstrated that the *ADC*1 locus is in the opposite orientation in pY9T5 and pY9T6 relative to the YRp7 vector sequences. The *Bam*HI site at the left end of pY9T6 is a cloning artefact produced by ligation of the *Sau*3A sticky end into the pBR322 *Bam*HI site. The pY9T5 insert is 7.2 kilobases and the pY9T6 insert 4.9 kilobases. Overlap between pY9T5 and pY9T6 defines a 4.1-kilobase sequence which is sufficient for ADHI expression.

```
            215                 220                 225
    Ile Gly Gly Glu Val Phe Ile Asp Phe Thr Lys Glu Lys Asp Ile
           • HphI
5'  ATC GGT GGT GAA GTC TTC ATT GAC TTC ACT AAG GAA AAG GAC ATT
3'  TAG CCA CCA CTT CAG AAG TAA CTG AAG TGA TTC CTT TTC CTG TAA

            230                 235                 240
    Val Gly Ala Val Leu Lys Ala Thr Asp Gly Gly Ala His Gly Val
                         HaeIII               HgiAI
    GTC GGT GCT GTT CTA AAG GCC ACT GAC GGT GGT GCT CAC GGT GTC
    CAG CCA CGA CAA GAT TTC CGG TGA CTG CCA CCA CGA GTG CCA CAG

            245                 250                 255
    Ile Asn Val Ser Val Ser Glu Ala Ala Ile Glu Ala Ser Thr Arg
                                Fnu4HI       HindIII(X)
    ATC AAC GTT TCC GTT TCC GAA GCC GCT ATT GAA GCT TCT ACC AGA
    TAG TTG CAA AGG CAA AGG CTT CGG CGA TAA CTT CGA AGA TGG TCT
                                              AluI

            260                 265                 270
    Tyr Val Arg Ala Asn Gly Thr Thr Val Leu Val Gly Met Pro Ala
             AluI        KpnI                            PvuII
    TAC GTT AGA GCT AAC GGT ACC ACC GTT TTG GTC GGT ATG CCA GCT
    ATG CAA TCT CGA TTG CCA TGG TGG CAA AAC CAG CCA TAC GGT CGA
                                                         AluI

            275                 280                 285
    Gly Ala Lys Cys Cys Ser Asp Val Phe Asn Gln Val Val Lys Ser
                                 MboII
    GGT GCC AAG TGT TGT TCT GAT GTC TTC AAC CAA GTC GTC AAG TCC
    CCA CGG TTC ACA ACA AGA CTA CAG AAG TTG GTT CAG CAG TTC AGG


            290                 295                 300
    Ile Ser Ile Val Gly Ser Tyr Val Gly Asn Arg Ala Asp Thr Arg

    ATC TCT ATT GTT GGT TCT TAC GTC GGT AAC AGA GCC GAC ACC AGA
    TAG AGA TAA CAA CCA AGA ATG CAG CCA TTG TCT CGG CTG TGG TCT

            305                 310                 315
    Glu Ala Leu Asp Phe Phe Ala Arg Gly Leu Val Lys Ser Pro Ile
     HindIII(Y)  MboII           MnlI
    GAA GCT TTG GAC TTC TTC GCC AGA GGT TTG GTC AAG TCT CCA ATC
    CTT CGA AAC CTG AAG AAG CGG TCT CCA AAC CAG TTC AGA GGT TAG
     AluI

            320                 325                 330
    Lys Val Val Gly Leu Ser Thr Leu Pro Glu Ile Tyr Glu Lys Met
                         AccI            EcoRI*
    AAG GTT GTC GGC TTG TCT ACC TTG CCA GAA ATT TAC GAA AAG ATG  3'
    TTC CAA CAG CCG AAC AGA TGG AAC GGT CTT TAA ATG CTT TTC TAC  5'
                                         EcoRI'
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Fig. 3 Partial DNA sequence of the *ADC*1 locus. The DNA sequence coding for amino acid residues 213–332 in the ADHI protein was determined by the technique of Maxam and Gilbert[23]. 100 µg of pY9T6 DNA was limit digested with *Hin*dIII then dephosphorylated with bacterial alkaline phosphatase and labelled with ^{32}P at the 5′ end using T4 polynucleotide kinase as described by Richardson[24]. DNA was then precipitated with ethanol to remove unreacted [γ-^{32}P]ATP. Half the labelled DNA was base denatured and loaded onto a long (38 cm) 6% native acrylamide gel. Electrophoresis for 18 h at 150 V resolved the separated strands of the 150-base pair restriction fragment between *Hin*dIII sites X and Y (Fig. 2). DNA fragments containing the other two pertinent labelled ends were isolated on 0.7% agarose gels, electroeluted, purified over DE-52, cleaved with a second restriction enzyme and resolved on another 0.7% agarose gel. Modifications and cleavages of the four electroeluted DNA samples were done by standard Maxam and Gilbert procedures[23]. Products were analysed on 8% and 20% acrylamide, 7 M urea sequencing gels which were either 0.5 mm or 0.7 mm thick. All but one of the 120 amino acids derived from the DNA sequence agree with the published amino acid sequence of the ADHI protein[14,15] for amino acid residues 213–332. Our sequence demonstrates that residue 236 is asparatic acid rather than asparagine. The restriction endonuclease sites shown were detected by scanning the derived DNA sequence. Only the *Hae*III and *Hin*dIII sites have been confirmed by actual restriction endonuclease digestion.

for the region surrounding *Hin*dIII sites X and Y (Fig. 3). Comparison of this sequence with the known amino acid sequence for ADHI[14] shows conclusively that these plasmids contain the structural gene. Figure 3 shows that the DNA sequence corresponds to an uninterrupted sequence encoding amino acids 213–332 in the ADHI protein with only one amino acid difference from the published sequence[14,15]. The extent of the coding sequence, if there are no intervening sequences, is indicated in Fig. 3. The ability of these plasmids to complement the growth defects of an ADH-null strain, together with the presence of normal levels of ADH activity, demonstrates that a functioning ADHI gene is present on both pY9T5 and pY9T6.

One reason for investigating ADH in yeast is to compare two genes which code for closely related proteins, ADHI and ADHII[15], but which are expressed in different conditions. As mentioned earlier, all the transformants which were selected for ADH activity contained ADHI, the constitutive enzyme. The absence of transformants containing ADHII was to be expected because the repression of this enzyme by the glucose present in our conditions of transformation would prevent it from complementing the growth defect of the recipient strain. The use of DNA from an available constitutive mutant of ADHII[4] should alleviate this problem and allow the ADHII structural gene (*ADR*2) to be cloned in a similar manner to *ADC*1. Alternatively, as ADHI and ADHII differ in only 15 of 284 amino acid positions[15], it is likely that their DNA sequences will cross-hybridise sufficiently to allow the already cloned ADC1 sequence to be used as a hybridisation probe for *ADR*2.

The use of complementation to isolate genes from a plasmid or phage pool is not new. Several yeast genes, for instance, *LEU*2, *HIS*3, *URA*3, and *TRP*1, have been isolated[11,16,17] by virtue of their ability to complement equivalent mutations in *E. coli*. By using yeast rather than *E. coli* cells as the recipient in transformation experiments, this same approach can be used to clone genes for which there is no analogous *E. coli* function. Preliminary experiments in our laboratory have shown that this potential is realisable for several different types of gene, for instance, those necessary for the cell division cycle (K. N. Nasmyth and S. I. Reed, in preparation) and mating. One might also speculate, based on the precedent set by the expression of yeast genes in *E. coli*, that yeast transformation may be a valuable technique for identifying and isolating genes from other eukaryotes. In particular, the system described here may prove useful for the isolation of ADH genes from other organisms.

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Trans-complementable copy-number mutants of plasmid ColE1

Andrea J. Twigg & David Sherratt

School of Biological Sciences, University of Sussex, Falmer, Brighton, UK

Plasmid ColE1, like many other small non-conjugative plasmids, is present in multiple copies (about 15 per chromsome equivalent) in *Escherichia coli* cells[1,2]. Because of their high copy number, the replication of such plasmids has been described as 'relaxed', even though there is good evidence that it is strictly controlled: ColE1 derivatives have characteristic but different copy numbers[2] and ColE1 copy-number mutants have been characterised[3–5]. No plasmid-specified protein is essential for the replication of ColE1 and related plasmids, as extensive replication can occur in chloramphenicol-treated cells[6], in plasmid-free chloramphenicol-treated cells transfected with a hybrid ColE1/phage replicon and *in vitro* in extracts derived from plasmid-free cells[8]. Nevertheless, it is possible that a plasmid-specified protein is involved in ColE1 replication control in viable cells. Here we show that deletion of a given non-essential region from ColE1-like plasmids results in a raised copy number. Such plasmids are stably maintained and have their copy number returned to normal when a complementing plasmid is present in the same cell, indicating that a plasmid-specified diffusible gene product regulates the plasmid content of ColE1-containing cells. Deletion of the equivalent region from the cloning vector pBR322 gives a derivative which has a raised copy number and which has also lost its origin for conjugal transfer; unlike pBR322, it cannot be mobilised.

Several chloramphenicol-resistant ($\mathrm{Cm^r}$) deletion derivatives of ColE1 were constructed by mixing partially *Hae*II-digested ColE1 DNA with a purified *Hae*II fragment, specifying $\mathrm{Cm^r}$, of pACYC184 (ref. 9). After ligation *in vitro*, the DNA was transformed into *E. coli* K12 [DS410 (ref. 10)], selecting for $\mathrm{Cm^r}$. The structure of some of the resulting plasmids is given in Fig. 1(*a*) and the plasmid DNA content of cells containing these mutants is shown in Table 1. For all mutants tested (including others not shown here), there is a direct correlation between raised plasmid level and the absence of the ColE1*Hae*II-C fragment. Mutants containing *Hae*II-C have a plasmid level close to that of ColE1, whereas those deleted for *Hae*II-C have a five to sevenfold increase in plasmid content. To determine whether *Hae*II-C contains all or part of a gene specifying a repressor for copy-number control and/or a site at which it acts, we attempted the complementation of raised copy number using the related plasmid, ColK. Although this plasmid is compatible with ColE1[11], the two plasmids show partial homology (D.S., unpublished) and have interchangeable mobility functions[11]. The effect of ColK and some of its deletion derivatives (Fig. 1*b*) on the ColE1 copy mutants is shown in Table 1. Whereas the presence of ColK reduced the plasmid level of all ColE1 copy mutants, the unrelated plasmid pSC101 and some of the ColK derivatives had little effect on ColE1 plasmid content. This suggests that we are observing true complementation rather than a nonspecific lowering of individual plasmid content. Examination of the structure of the ColK plasmids that have no effect on the ColE1 copy mutants indicates that the complementing gene product (repressor) is specified by a gene whose expression to give an active gene product disappears when ColK*Hae*II-B is deleted; the gene could be wholly contained on the fragment or extend into the E or even C fragment of ColK. This region corresponds in position and is probably partially homologous to the *Hae*II-C fragment of ColE1, thus supporting the idea that this region in both ColE1 and ColK encodes a repressor of plasmid replication. The site of action of this repressor must be present in all the copy mutants.

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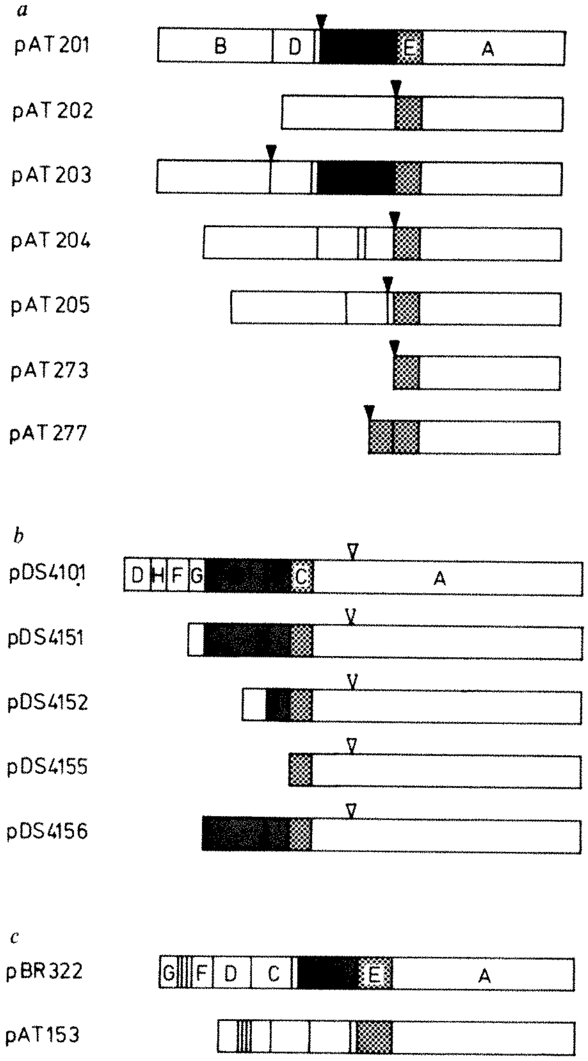

Fig. 1 Structures of the plasmids used. *a*, Cm^{r} ColE1 derivatives. pAT201, and pAT203 have the entire ColE1 genome with the Cm^{r} *Hae*II fragment of pACYC184[9] (▼) inserted at either of two *Hae*II sites; the other plasmids have various ColE1 *Hae*II fragments deleted. *b*, Ap^{r} ColK derivatives. pDS4101 is ColK: : Tn1. The other plasmids are deletion derivatives of this. ▽ Indicates intact Tn1, and V indicates that some of the Tn1 internal *Hae*II fragments have been deleted. *c*, pBR322 and its *Hae*II deletion derivative pAT153. Vertical lines indicate *Hae*II sites. Light stippling indicates the *Hae*II fragment carrying the origin of replication and heavy stippling indicates the region encoding the gene product (repressor) that maintains normal plasmid copy number.

Table 2 Conjugal mobility of plasmid cloning vectors

Plasmid vector	Mobilisation promoted by conjugative plasmid R64*drd*11*	Mobilisation from a R64 *drd*11 ColK† cell
pBR322	$<8\times10^{-6}$	9×10^{-2}
pAT153	$<1\times10^{-5}$	$<4\times10^{-6}$
pACYC184	$<9\times10^{-6}$	$<2\times10^{-5}$

* Frequency of R64-receiving recipients that inherit given factor.

† ColK is a natural relative of ColE1 that specifies *trans*-acting mobility proteins that are interchangeable with those of ColE1 for R64*drd*11-promoted transfer[11].

To test if deletion of the equivalent region from plasmids derived from natural relatives of ColE1, results in increased copy number, we partially digested with *Hae*II the cloning vector pBR322 (refs 12, 13), which is ampicillin resistant (Ap^{r}) and tetracycline resistant (Tc^{r}) and is derived from pMB1, closely related to ColE1[14]. Inspection of the published sequence of the pBR322 *Hae*II-B fragment[13] with that available to us for the ColE1*Hae*II-C fragment, shows that most of pBR322 *Hae*II-B is contained within ColE1*Hae*II-C in a highly conserved form (also see ref. 13, page 85). After *in vitro* ligation and transformation, selecting for Ap^{r}Tc^{r}, we isolated a deletion derivative lacking the 0.62-kilobase *Hae*II-B fragment of pBR322 (Fig. 1*c*). Cells containing this plasmid (pAT153) have a 1.5–3-fold greater plasmid content than those containing the parental plasmid. Although this increase is smaller than that for the ColE1 deletion derivatives, we believe that it makes pAT153 a potentially useful cloning vector because of greater plasmid and plasmid gene product yields per ml of culture. pAT153, like pBR322, replicates in chloramphenicol-treated cells. In terms of biological containment for *in vitro* genetic manipulation, pAT153 also has a great advantage over pBR322 in that it has lost *nic*, the transfer origin for conjugal transfer. Whereas pBR322 is efficiently mobilised from cells containing the appropriate conjugative plasmid and ColE1-like mobility proteins, no transfer of pAT153 has been observed (Table 1). It can also be seen that pACYC184 behaves as a *nic*$^{-}$ plasmid. Similarly, ColE1 deletions lacking *Hae*II-C are non-mobilisable because they lack *nic*[15]. The increased gene-dosage effects resulting from the increased copy number of the ColE1 deletion derivatives have also been detected by the increased antibiotic resistance conferred on plasmid-containing cells and by the increased frequency of spontaneous colicin production by cells containing colicin-producing copy mutants (A.J.T., unpublished).

Table 1 Plasmid content of cells containing Cm^{r} ColE1 deletion mutants

		ColE1 plasmid content (as % of chromosomal DNA) when:							
ColE1 derivative	Size (kilobases)	No other plasmid present	ColK present	pSC101 present	pDS4101 present	pDS4155 present	pDS4151 present	pDS4152 present	pDS4156 present
ColE1	6.3	3.8 (0.92)	–	–	–	–	–	–	–
pAT201	7.8	2.8 (0.44)	–	–	–	–	–	–	–
pAT202	5.4	13.9 (2.1)	2.1 (0.31)	–	–	–	–	–	–
pAT203	7.8	4.5 (0.96)	–	–	–	–	–	–	–
pAT204	6.9	16.5 (1.2)	2.9 (0.53)	13.0 (0.98)	0.7 (0.10)	12.0 (1.4)	2.5 (0.38)	20.9 (3.1)	0.8 (0.20)
pAT205	6.2	17.7 (2.7)	1.4 (0.60)	–	1.4 (0.53)	13.7 (0.96)	0.8 (0.23)	15.0 (1.8)	0.5 (0.15)
pAT273	3.9	20.5 (1.8)	1.9 (0.32)	13.6 (1.6)	–	–	–	–	–
pAT277	4.3	20.6 (2.0)	1.4 (0.28)	19.9 (2.2)	0.5 (0.21)	12.2 (1.6)	2.1 (0.29)	18.8 (1.1)	0.5 (0.24)

Plasmid content values are means of at least three determinations carried out as described in Fig. 2 legend. These values can be easily converted to copy number per genome equivalent (3.8×10^{3} kilobase pairs). Values in parentheses are standard deviations. –, Not determined.

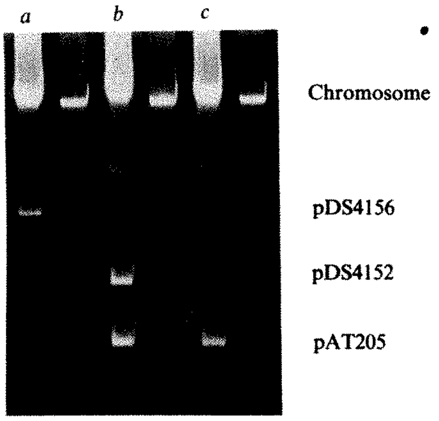

Fig. 2 Copy-number determination. Representative 1% agarose gel electrophoresis of total cell lysates. Stationary phase cells (about one colony equivalent) from either selective agar or a liquid culture were resuspended in 250 μl of 40 mM Tris acetate, 20 mM sodium acetate, 1 mM disodium EDTA (*p*H 8.2) electrophoresis buffer containing 1% SDS, 2% Ficoll and 0.01% bromophenol blue. After heating at 65° C for 30 min and vortex mixing, 50 μl each of undiluted and 10-fold diluted sample were applied to alternate gel wells. After running (50 V for 4 h) and staining with ethidium bromide (0.5 μg ml^{-1}, 60 min), the UV-fluorescence of the gel was photographed using a range of exposures. Microdensitometer tracings of suitable photographic negatives allowed the relative proportions of plasmid and chromosomal DNA to be determined for each lysate. This gel shows the results of copy-number complementation by ColK derivatives. *a*, pDS4156+pAT205; *b*, pDS4152+pAT205; *c*, pAT205 alone. The fainter alternate tracks are the respective 10-fold diluted samples. Although most of our copy-number determinations were made on stationary phase cells, the same relative differences between ColE1 and its copy mutants were observed in exponentially growing cells.

The presumptive replication repressor described above seems not to be intimately involved in determining plasmid incompatibility[16,17], the phenomenon that prevents two closely related replicons stably coexisting within a cell. ColE1 and ColK have cross-reacting repressors, but are completely compatible, whereas two ColE1 derivatives lacking *Hae*II-C are not stably co-maintained. Moreover, other ColE1 sequences are involved in determining plasmid copy number. For example, an insertion of some 30 base pairs in part of the ColE1 region essential for autonomous replication results in a copy-number phenotype[4,5]. The RNA transcript of the region seems to be important in specifying copy number[5]. It will be of interest to study the interactions of the two types of copy-number mutant.

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Excision sequences in the mitochondrial genome of yeast

Claire Gaillard, François Strauss & Giorgio Bernardi

Laboratoire de Génétique Moléculaire,
Institut de Recherche en Biologie Moléculaire, Paris, France

It is well established that spontaneous cytoplasmic 'petite' mutants of *Saccharomyces cerevisiae* have mitochondrial genome units in which an excised segment of the parental wild-type genome has been tandemly amplified (Fig. 1), so that the excised segment becomes the repeat unit of the petite genome; the latter may in turn undergo further deletions leading to secondary petite genomes having shorter repeat units (see ref. 1 for a brief review). Recent investigations[2] on the mitochondrial genomes of several spontaneous petite mutants have shown that frequently the ends of the excised segment correspond to short sequences of the wild-type genome which are extremely rich in GC, the GC clusters; alternatively, they seem to be located in the long AT-rich stretches, the AT spacers, which form at least half of the genome. As sequence repetitions have been demonstrated in both GC clusters and AT spacers[2–5], it is very likely that excision takes place by a mechanism involving illegitimate site-specific recombination events between homologous sequences, as previously postulated[1]. We show here that the sequences involved in the excision of a particular spontaneous petite genome are direct nucleotide repeats located in the AT spacers.

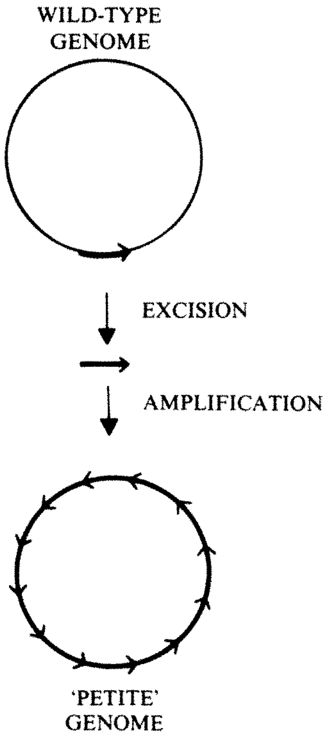

Fig. 1 Scheme of the process leading to the formation of spontaneous petite genomes. A segment of a mitochondrial genome unit from wild-type yeast cells is excised and tandemly amplified to yield the mitochondrial genome unit of a petite mutant. The excised segment from the wild-type genome becomes the repeat unit of the petite genome. This may in turn undergo further deletions leading to secondary petite genomes having simpler repeat units.

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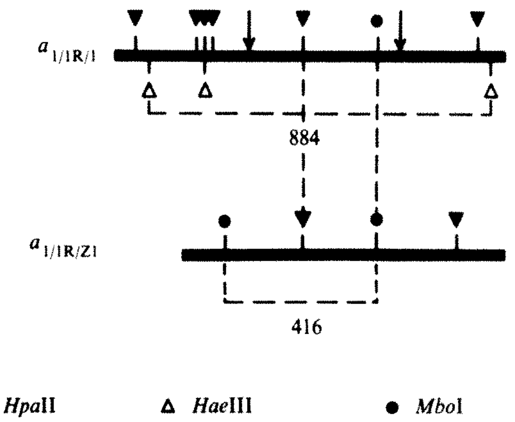
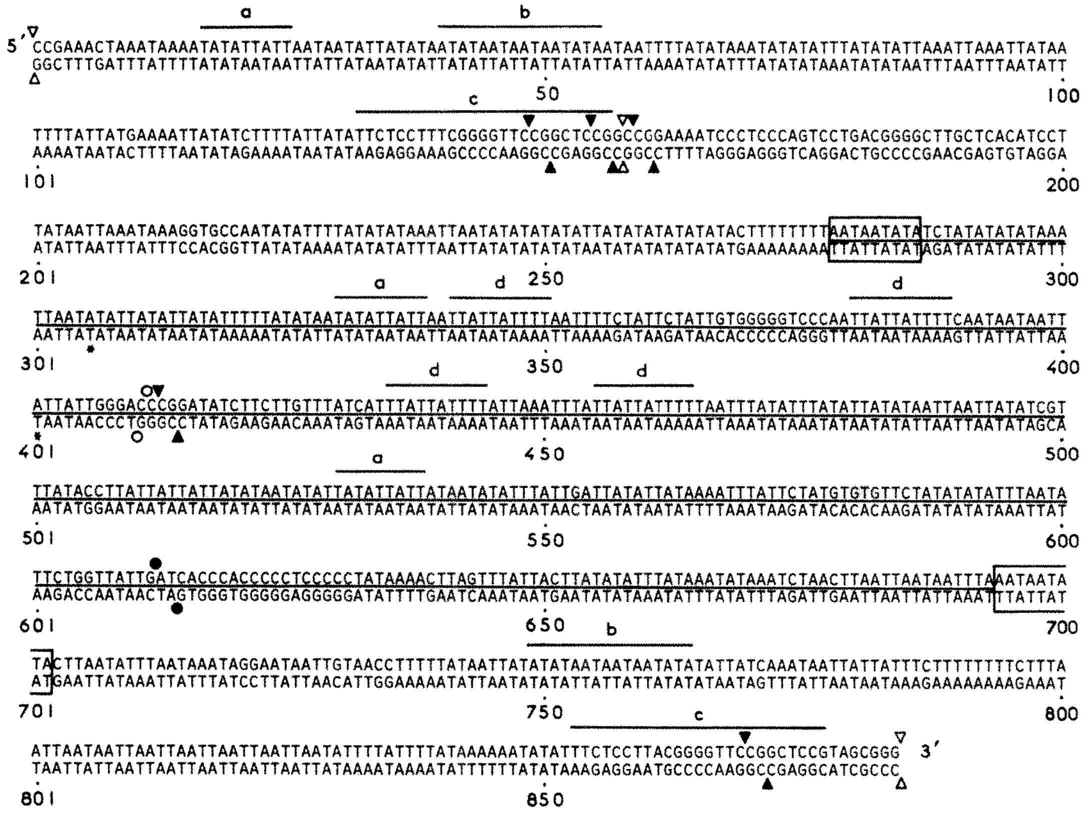


Fig. 2 Restriction enzyme maps of the repeating units of the mitochondrial genomes of two spontaneous petite mutants. The molecular weights of the repeat units are indicated (horizontal broken lines), along with the positions of *Hae*III (Δ), *Hpa*II (▼) and *Mbo*I(●) restriction sites. The vertical broken lines indicate corresponding restriction sites in the repeat units of the two genomes. Arrows indicate the approximate positions of excision sites as obtained from the data of Fig. 3.

Our approach was to sequence the repeat unit of the mitochondrial genome of a spontaneous petite mutant, $a_{1/1R/1}$, which was supposed to contain the repeat unit and the flanking sequences of another spontaneous petite mutant, $a_{1/1R/Z1}$. Both petite genomes had been previously characterised[2]; they originated from the same region of the parental wild-type mitochondrial genome (between map positions 27 and 46, ref. 6) and their restriction maps were known (Fig. 2); the DNA from $a_{1/1R/Z1}$ hybridised on restriction fragments from wild-type DNA corresponding to the larger *Hae*III fragments and to the two largest *Hpa*II fragments of $a_{1/1R/1}$, and shared a *Hpa*II–*Mbo*I fragment with $a_{1/1R/1}$; finally, the sequence of the repeat unit of $a_{1/1R/Z1}$ was known[5].

The nucleotide sequence was determined, using the method of Maxam and Gilbert[7], on both mitochondrial DNA prepared from $a_{1/1R/1}$ and its *Mbo*I repeat unit inserted in the *Bam*HI site of pBR322 plasmid and amplified in a *rec*$^+$ *Escherichia coli* strain (HVC46; C600 *leu*$^-$, *thi*$^-$, *lac*$^-$, *tonA*, *supE*44, *hsdR*, *hsdM*). The same sequence was found in both cases (Fig. 3). The repeat unit of $a_{1/1R/1}$ is 884 nucleotides long and contains, between positions 278 and 693, a 416-nucleotide segment which is identical to the sequence of the repeat unit of $a_{1/1R/Z1}$ (ref. 5), except for two base pair changes, which are indicated by an asterisk in Fig. 3. The most important feature of the sequence of $a_{1/1R/1}$ is that the initial nonanucleotide of the repeat unit of $a_{1/1R/Z1}$, AATAATATA, is repeated just after the end of the repeat unit; if allowance is made for one A·T to T·A change, these direct repeats are, in fact, 13 nucleotides long. This situation is reminiscent of those found on both sides of insertion sequence IS*1* and of transposon Tn*9* in *E. coli*, where two direct repeats of 9 base pairs were found[8,9]. It provides evidence consistent with the excision model proposed previously, and raises the interesting possibility that segments like $a_{1/1R/Z1}$ can be transposed onto other mitochondrial genome units and contribute to the changes in length observed in genome units belonging to different strains[10]. Incidentally, the data do not allow one to decide whether the repeat unit of $a_{1/1R/Z1}$ was excised from the parental wild-type genome or from $a_{1/1R/1}$, although the latter explanation seems the more likely. This is not an essential point, however, as it is known that the same excision process is operative in wild-type and petite genomes[2].

Several other features of the sequence of $a_{1/1R/1}$ are worth mentioning: (1) two direct AT repeats, 16 nucleotides long, containing the nonanucleotides flanking the $a_{1/1R/Z1}$ sequence,



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5' CCGAAACTAAATAAAATATATTATTAATAATATTATATAATATAATAATAATATAATAATTTTATATAAATATATATTTATATATTAAATTAAATTATAA
   GGCTTTGATTTATTTTATATAATAATTATTATAATATATTATATTATTATTATATTATTAAAATATATTTATATATAAATATATAATTTAATTTAATATT
                                                                                                       100
   TTTTATTATGAAAATTATATCTTTTATTATATTCTCCTTTCGGGGTTCCGGCTCCGGCCGGAAAATCCCTCCCAGTCCTGACGGGGCTTGCTCACATCCT
   AAAATAATACTTTTAATATAGAAAATAATATAAGAGGAAAGCCCCAAGGCCGAGGCCGGCCTTTTAGGGAGGGTCAGGACTGCCCCGAACGAGTGTAGGA
   101                                                                                                 200
   TATAATTAAATAAAGGTGCCAATATATTTTATATATAAATTAATATATATATATTATATATATATATACTTTTTTTAATAATATATCTATATATATAAA
   ATATTAATTTATTTCCACGGTTATATAAAATATATATTTAATTATATATATATAATATATATATATATGAAAAAAATTATTATATAGATATATATATTT
   201                                                                                                 300
   TTAATATATTATATTATATTTTTATATAATATATTATTAATTATTATTTTAATTTTCTATTCTATTGTGGGGGTCCCAATTATTATTTTCAATAATAATT
   AATTATATAATATAATATAAAAATATATTATATAATAATTAATAATAAAATTAAAAGATAAGATAACACCCCCAGGGTTAATAATAAAAGTTATTATTAA
   301                                                                                                 400
   ATTATTGGGACCCGGATATCTTCTTGTTTATCATTTATTATTTTATTAAATTTATTATTATTTTTAATTTATATTTATATTATATAATTAATTATATCGT
   TAATAACCCTGGGCCTATAGAAGAACAAATAGTAAATAATAAAATAATTTAAATAATAATAAAAATTAAATATAAATATAATATATTAATTAATATAGCA
   401                                                                                                 500
   TTATACCTTATTATTATTATATAATATATTATATTATTATAATATATTTATTGATTATATTATAAAATTTATTCTATGTGTGTTCTATATATATTTAATA
   AATATGGAATAATAATAATATATTATATAATATAATAATATTATATAAATAACTAATATAATATTTTAAATAAGATACACACAAGATATATATAAATTAT
   501                                                                                                 600
   TTCTGGTTATTGATCACCCACCCCCTCCCCCTATAAAACTTAGTTTATTACTTATATATTTATAAATATAAATCTAACTTAATTAATAATTTAAATAATA
   AAGACCAATAACTAGTGGGTGGGGGAGGGGGATATTTTGAATCAAATAATGAATATATAAATATTTATATTTAGATTGAATTAATTATTAAATTTATTAT
   601                                                                                                 700
   TACTTAATATTTAATAAATAGGAATAATTGTAACCTTTTTATAATTATATATAATAATAATATATATTATCAAATAATTATTATTTCTTTTTTTTCTTTA
   ATGAATTATAAATTATTTATCCTTATTAACATTGGAAAAATATTAATATATATTATTATTATATATAATAGTTTATTAATAATAAAGAAAAAAAAGAAAT
   701                                                                                                 800
   ATTAATAATTAATTAATTAATTAATTAATATTTTATTTTATAAAAAATATATTTCTCCTTACGGGGTTCCGGCTCCGTAGCGGG 3'
   TAATTATTAATTAATTAATTAATTAATTATAAAATAAAATATTTTTTATATAAAGAGGAATGCCCCAAGGCCGAGGCATCGCCC
   801
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Fig. 3 Nucleotide sequence of the repeat unit of the mitochondrial genome of spontaneous petite mutant $a_{1/1R/1}$. Cutting sites of *Hae*III (Δ), *Hpa*II (▲), *Mbo*I (●) and *Mbo*II (○) are indicated. The sequence between positions 278 and 693 is identical to that of $a_{1/1R/Z1}$ (ref. 15) except for asterisked base pairs 306 and 401, which are replaced by G·C and C·G, respectively, in $a_{1/1R/Z1}$. Boxed sequences correspond to the nonanucleotides flanking the sequence of $a_{1/1R/Z1}$. Lines indicate repeated sequences (see text).

are found at positions 40–55 and 749–764 (these are labelled b in Fig. 3); (2) two GC-rich repeats, 25 nucleotides long with only one A·T to T·A change, are found at positions 132–156 and 853–877 (these are labelled c in Fig. 3); the first one is part of a 65-nucleotide stretch very rich in GC (GC = 65%), which accounts for the higher GC level of $a_{1/1R/1}$ (GC = 16.2%) compared with $a_{1/1R/Z1}$ (GC = 14.2%); (3) a 20-nucleotide sequence formed by five repeats of the sequence TTAA is found at positions 809–828; (4) symmetrical sequences are found in the GC cluster at position 150, in agreement with previous predictions[10] and findings[3,4]; (5) other sequence features of $a_{1/1R/1}$ are common with $a_{1/1R/Z1}$ and have already been mentioned[5]; the most remarkable one is a 47-nucleotide stretch (369–415) formed by a palindrome 23 nucleotides long (378–400) and a small symmetrical sequence TTATT (402–406), which are flanked by two symmetrical GC clusters (369–377 and 407–415).

The main conclusion of the present work is that crossing-over involving short homologous sequences is indeed the most likely mechanism underlying the excision of spontaneous petite genomes. Sequences used in the excision of other spontaneous petite genomes are being investigated in our laboratory. Some of the genomes under consideration are also encompassed by $a_{1/1R/1}$, and it will be interesting to see which other sequences of $a_{1/1R/1}$ are involved in excision. We expect to find among them some or the other repeats of $a_{1/1R/1}$.

Another point of interest is that the presence of direct repeats in its sequence has not caused any instability of the $a_{1/1R/1}$ repeat unit as propagated in a *rec*$^+$ *E. coli*; it should be stressed, however, that monomers of the repeat unit were cloned and that oligomers were not tested.

Finally, an inspection of the sequence of $a_{1/1R/1}$ fails to reveal the presence of any gene, a conclusion confirmed by the present knowledge of codons used for the synthesis of mitochondrially encoded proteins[4,11] and of tRNA gene sequences[12]. The only function left in this petite genome (as well as in $a_{1/1R/Z1}$) is replication. As the excision process leading to the formation of spontaneous petite genomes is extremely conservative as far as the excised sequence is concerned[2] (in contrast to that induced by ethidium, where sequence rearrangements are frequent [13]), each repeat unit of $a_{1/1R/1}$ should contain a site used for the initiation of DNA replication. A good candidate is the 80-nucleotide sequence centred around position 412. This sequence, (which contains the remarkable stretch, 369–415, mentioned above), is present not only in the two overlapping genomes $a_{1/1R/1}$ and $a_{1/1R/Z1}$, but also in another one[14], b, whose 875-nucleotide repeat unit is derived[2] from an entirely different region of the parental wild-type genome (map positions 64–76, ref. 6). If a site for the initiation of DNA replication is present in each repeat unit of these petite genomes, a high replication rate may be expected. This indeed seems to be the case, as the three petites under consideration are 'supersuppressive', namely petites whose mitochondrial genomes compete out those of wild-type cells in crosses and become the only ones transmitted to the progeny[14].

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Are snRNPs involved in splicing?

Michael R. Lerner, John A. Boyle, Stephen M. Mount, Sandra L. Wolin & Joan A. Steitz

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06510

Discrete, stable small RNA molecules are found in the nuclei of cells[1] from a wide variety of eukaryotic organisms[2]. Many of these small nuclear RNA (snRNA) species, which range in size from about 90 to 220 nucleotides, have been well-characterised biochemically[3–6], and some sequenced[7,8]. However, their function has remained obscure. The most abundant snRNA species exist as a closely related set of RNA–protein complexes called small nuclear ribonucleoproteins (snRNPs)[9]. snRNPs are the antigens recognised by antibodies from some patients with lupus erythematosus (LE), an autoimmune rheumatic disease[10,11]. Anti-RNP antibodies from lupus sera selectively precipitate snRNP species containing Ula[7] and Ulb[9] RNAs from mouse Ehrlich ascites cell nuclei, whereas anti-Sm antibodies bind these snRNPs and four others containing U2 (ref. 8), U4, U5 and U6 (ref. 9) RNAs. Both antibody systems precipitate the same seven prominent nuclear proteins (molecular weight 12,000–32,000). All molecules of the snRNAs U1, U2, U4, U5 and U6 appear to exist in the form of antigenic snRNPs[9]. The particles sediment at about 10S and each probably contains a single snRNA molecule. Indirect immunofluorescence studies (refs 12, 13, and unpublished observations) using anti-RNP and anti-Sm sera confirm the nuclear (but non-nucleolar) location of the antigenic snRNPs. Here we present several lines of evidence that suggest a direct involvement of snRNPs in the splicing of hnRNA. Most intriguing is the observation that the nucleotide sequence at the 5′ end of U1 RNA exhibits extensive complementarity to those across splice junctions in hnRNA molecules.

If snRNPs participate in RNA processing, they might be expected to be strictly conserved across those higher eukaryotic species possessing interchangeable transcription and mRNA processing systems (refs 14–17 and P. Chambon, personal communication). Figure 1 shows that the antigenic snRNPs are indeed highly conserved from man to insects. The anti-RNP lanes reveal that mouse snRNPs containing the closely related Ula and Ulb RNAs[9] are replaced by a single U1-containing snRNP in HeLa and fall armyworm cells. Fingerprints (not shown) indicate that the sequence of HeLa U1 RNA is identical to that of mouse U1a[9] and to that of rat U1a[7]; insect U1 RNA has a quite different fingerprint, although it is comparable in size to the mammalian U1. Likewise, when anti-Sm antibody is used, four additional HeLa or insect cell snRNPs that contain RNAs very close in mobility to mouse U2, U4, U5 and U6 are precipitated; again, fingerprints of the HeLa U2, U4, U5 and U6 RNAs are identical to those of mouse(not shown). Also, human antibodies precipitate snRNPs from the nuclei of frog, chicken and sea urchin cells, indicating high conservation of both RNA and protein components of snRNPs in these species as well. No cross-reacting material is detected in tobacco cell, yeast, *Dictyostelium discoideum* or *Escherichia coli* extracts.

A second prediction concerning snRNP involvement in RNA biogenesis is that snRNAs should be present in highest abundance in metabolically active cell types. Figure 2 compares nuclear extracts from liver and from red blood cells (RBC) of chickens. The sucrose gradient profiles (Fig. 2*a*) showing extracts from equal numbers of the two types of nuclei reveal fewer than one-tenth as many 30S hnRNP particles (the highly conserved ribonucleoprotein complexes that bind hnRNA in the nuclei of higher eukaryotes[18–20]) and much less RNA sedimenting in the 10S region in the case of the RBC. Also, gel analysis of

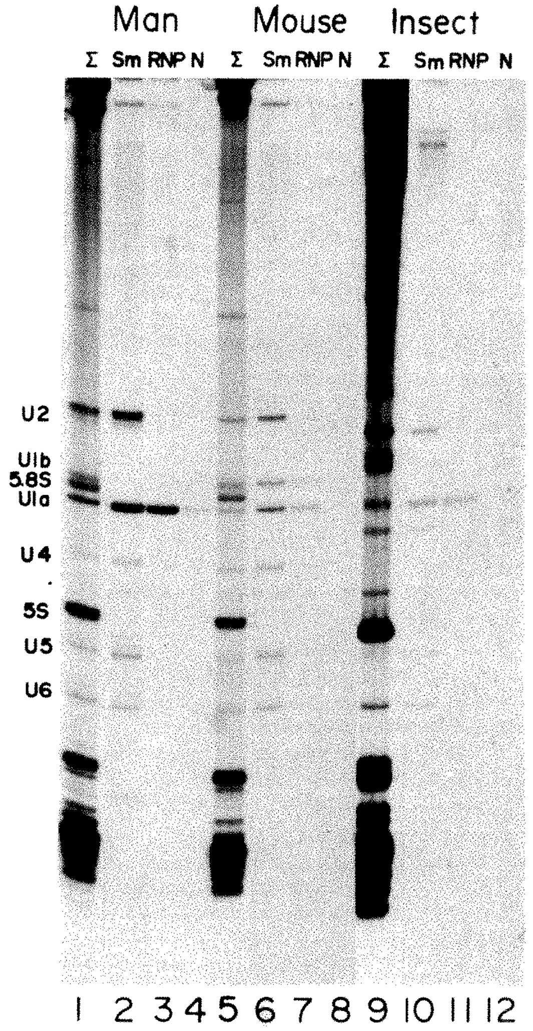

Fig. 1 Comparison of snRNPs from different species. Small RNAs from cell sonicates or immune precipitates from HeLa (lanes 1–4), Ehrlich ascites (lanes 5–8) and fall armyworm (lanes 9–12) cells were prepared as previously described[9] using Pansorbin to obtain the antigen–antibody complexes. The gel contained 10% polyacrylamide, 7 M urea, 1 mM EDTA, 50 mM Tris-borate *p*H 8.3 and was 400×200×0.5 mm. Lanes 1, 5 and 9 show small RNAs in whole cell extracts; 2, 6 and 10 show typical anti-Sm patterns; while 3, 7 and 11 show typical anti-RNP patterns. Lanes 4, 8 and 12 are controls using normal serum.

nuclear RNAs reveals snRNAs in both cases, but about 25-fold fewer in liver than in RBC (Fig. 2*b*, compare lane 2 with lanes 3 and 4). Treatment of the chicken with phenylhydrazine, which raised the level of erythroblasts to about 25% of the total RBC, altered the snRNA gel pattern (not shown) to that characteristic of liver cells (Fig. 2*b*, lane 2). Thus, liver cells, which actively synthesise mRNA, have both higher amounts and a slightly different population of snRNAs than the cryptic nuclei of biosynthetically inactive erythrocytes. Were the result otherwise, it would constitute evidence against snRNP participation in RNA processing.

Finally, a number of workers[4,21-24] have observed snRNAs co-sedimenting with larger nuclear structures, such as the 30S particles that bind hnRNA. We have argued[9] that the snRNAs U1, U2, U4, U5 and U6 are not structural components of 30S hnRNP. Yet, as shown in Fig. 3*a*, *b*, on sucrose gradient fractionation of a nuclear extract prepared from Ehrlich ascites cells, antigenic snRNPs containing these snRNA species do sediment in the 30S region of the gradient. An RNA-labelled U1a*, however, appears only in the 10S region where the free snRNP particles sediment. Fingerprint analysis (Fig. 3*c*) reveals that U1a* is identical to U1a (Fig. 3*d*) except that it lacks the 5′ trimethylated cap moiety plus six additional nucleotides from the 5′ end of the U1a sequence. Further, U1a* is not present in fresh extracts and the RNA–protein complex containing U1a* is fully antibody precipitable, strongly suggesting that U1a* is an *in vitro* degradation product of U1a. Thus, the absence of a short region at its 5′ terminus seems to prevent the U1a-containing snRNP from co-sedimenting with larger structures containing hnRNA.

It is interesting that several of the residues missing in U1a* fall within a region of U1a which is extensively complementary to sequences across splice junctions in hnRNAs (Fig. 4). DNA sequences corresponding to the ends of 43 introns were analysed (Table 1). Redundant sequences (those which are identical and occur in the same position on homologous RNAs) were discarded, leaving 26 unique sequences at the 5′ ends and 31 unique sequences at the 3′ ends of introns. The two consensus splice junction sequences are presented in Fig. 4*a*; in each position, the frequency of occurrence of the most common base is indicated by underlining as described in the legend. Potential base-pairing interactions between these regions and the nucleotide sequence at the 5′ end of U1a[7] or U1b RNA[9] are also shown. Note that the complementarity of U1 to the consensus sequence at the 5′ end of introns involves eight contiguous residues, plus an adjacent nucleotide (three bases before the splice point) which is generally either C or A. (It seems reasonable to consider that the $\overset{A}{C}$ position is also complementary since A·G base pairs are observed where the two strands of an RNA helix diverge in yeast tRNA^{Phe} (ref. 25).) Of the 26 5′ junction sequences, all but one has the dinucleotide GU immediately adjacent to the splice point and at least five (usually six) bases of the consensus sequence. Similarly, at the 3′ end of introns, all but one of the 31 unique sequences has the dinucleotide AG, and all

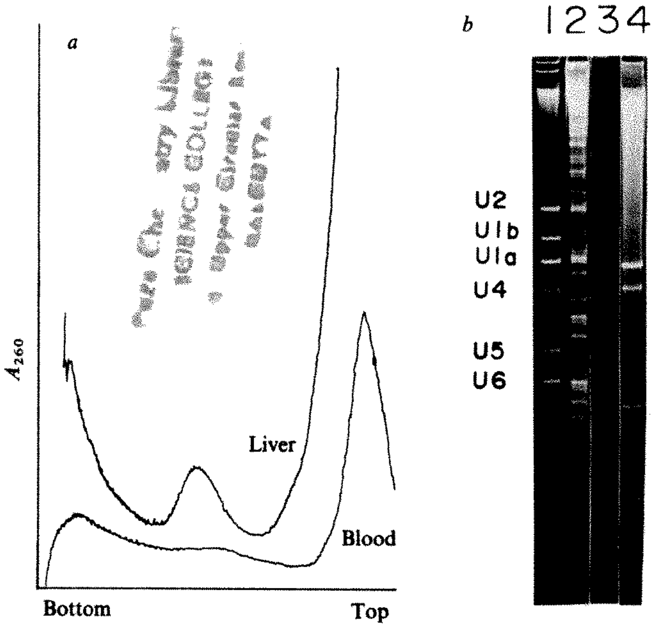

Fig. 2 Sedimentation patterns and gel analysis of small RNAs from nuclear extracts of liver and red blood cells of an adult chicken. *a*, Comparable numbers of liver and RBC nuclei (prepared as in refs 18 and 9, respectively) were extracted with *p*H 8.5 buffer[18]. The extracts were applied to 15–30% sucrose gradients and centrifuged for 17 h at 4 °C and 23,000 r.p.m. in an SW 41 rotor. SDS polyacrylamide gel electrophoresis[9] confirmed that the 30S peak contains primarily the prominent proteins of the 30S hnRNP[18-20]. *b*, RNAs obtained by phenol extraction were analysed by electrophoresis as in Fig. 1. Lane 1, marker Ehrlich ascites snRNAs selected by immunoprecipitation with anti-Sm antibody. Lane 2, total RNA from an extract of chicken liver nuclei. Lane 3, total RNA from an extract of an equivalent number of chicken RBC nuclei. Lane 4, total RNA from an extract of chicken RBC nuclei; the amount of extract loaded represents a 25-fold increase in number of nuclei over that shown in lanes 2 and 3.

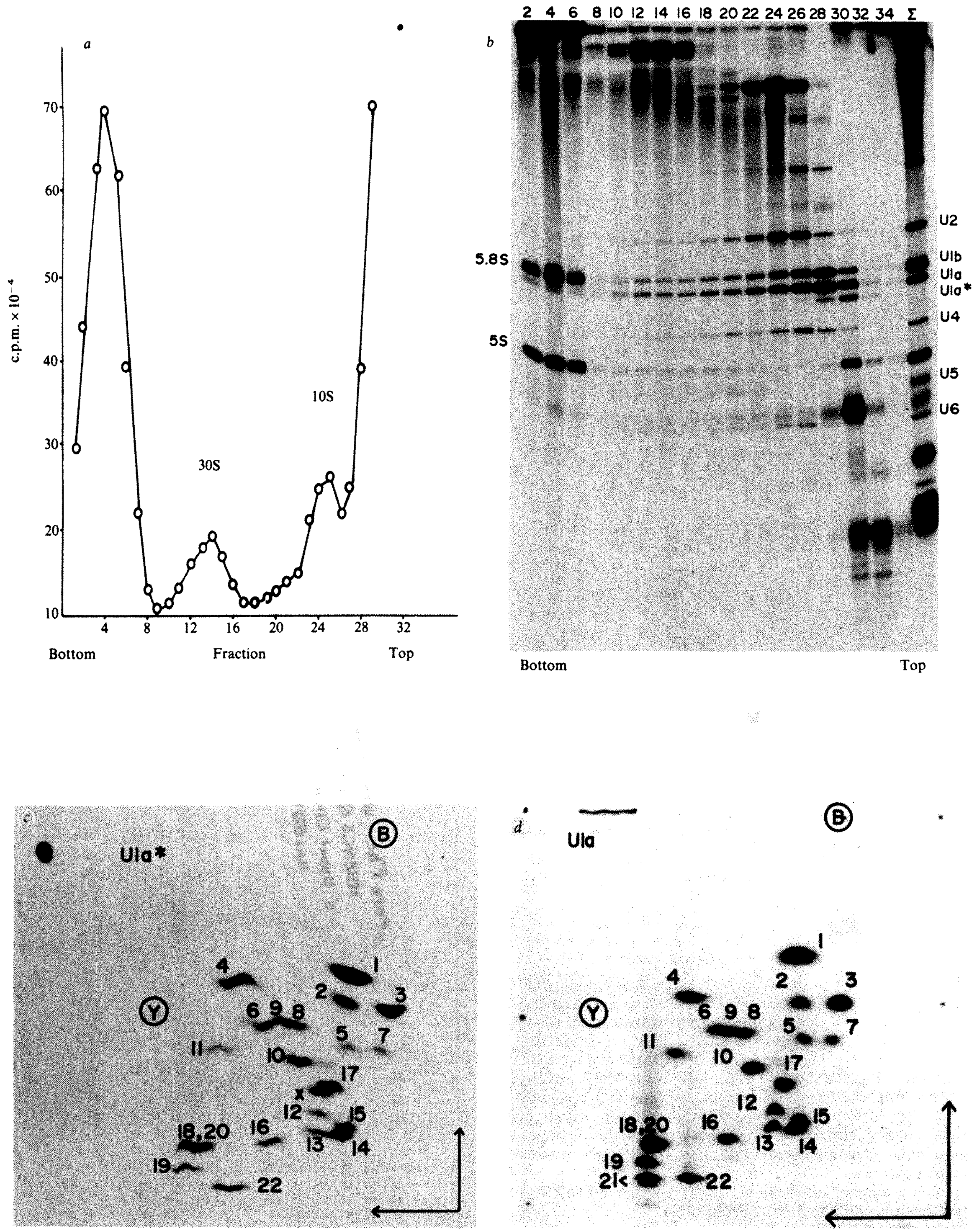

Fig. 3 Location and identification of snRNAs after sucrose gradient fractionation of a nuclear extract. *a*, Nuclei were prepared from ^{32}P-labelled Erlich ascites cells, extracted in high *p*H buffer[9] and the extract applied to a sucrose gradient as in Fig. 2*a*, *b*. The lanes in the gel (run as in Fig. 1) correspond directly to the numbered fractions in the gradient. Direct phenol extraction and prior immunoprecipitation with anti-Sm serum revealed the same distribution of snRNAs. Some contaminating ribosomes appear near the bottom of the gradient, but SDS polyacrylamide gel analysis reveals that the 30,000–40,000 MW 'core proteins'[18–20] of the hnRNP predominate in the 30S region. Indicated RNA species were identified by fingerprint analysis. *c*, T1 RNase fingerprint of U1a. The numbering system corresponds to ref. 7. Ⓑ and Ⓨ indicate the positions of the blue and yellow dyes in first (horizontal) and second (vertical) dimension. Spot 21 is the 5′ end oligonucleotide. *d*, T1 RNase fingerprint of U1a*. Spot X is the remnant of the 5′ oligonucleotide.

Table 1 Intron–exon boundary sequences

Gene	5′Exon|	Intron |Exon 3′	
Rat insulin	CAGGUAUGU	··· CUAUCUUCCAGG	36†
Rat insulin	AAGGUAAGC	··· CUCCCUGGCAGU	36
Rat insulin	CAGGUAUGU	··· CUAUCUUCCAGG	36
γ1 chain (newborn mouse)		··· UUUUCUUGUAGC	37
γ1 chain (newborn mouse)	UUGGUGAGA	··· UCUCUCCACAGU	37
	CAGGUAAGU	UUCAUCCUUAGU	
γ1 chain (newborn mouse)	AAGGUGAGA	··· CCCACCCACAGG	37
		UUUUCUUGUAGC	
γ1 chain (mouse myeloma)	UUGAGAGGA	··· UCUCUCCACAGU	38
γ2 chain (mouse myeloma)	CAGGUAAGU	··· UUCAUCCUUAGU	38
	AAGGUGAGA	CUCACUCACAGG	
γ1 chain (mouse myeloma)	CAGGUCAGC	··· CCUGUUUGCAGG	38
	CAGGUCAGC	UCUGUUUGCAGG	
γ1 chain (mouse myeloma)	UAGGUGAGU	··· UCAUCCUGCGGC	38
γ2 Chain	AACGUAAGU	UCCUUCCUCAGG	39
λ1 Chain	AACGUAAGU	··· UCCUUCCUCAGG	40
λ1 Chain	AACCUAAGU	··· UCCUUCCUCAGG	40
κ Chain	AACGUAAGU	··· UCCUUCCUCAGG	41
κ Chain	AACGUAAGU	··· UCCUUCCUCAGG	41
κ Chain	AAGGUUAAA	··· UCCACUCCUAGG	41
κ Chain	CAGGUUGGU	··· UCCCUUUUUAGG	41
κ Chain	AGGGUGAGU	··· UAUUCCCACAGC	41
κ Chain Mouse β-globin	CAGGUUGGU	··· CAUUUUCUCAGG	42 43
Mouse β-globin	AGGGUGAGU	··· UUUUCCUACAGC	43
Rabbit β-globin		··· UCCUCCCACAGC	43
Rabbit β-globin		··· CUUCUCCGCAGC	43
Human β-globin	AAGGUAGGC	··· GUUUGCUCUAGA	43
Human δ-globin	AAGGUGAGC	··· UUCAAUUACAGG	44
Chicken ovalbumin Chicken ovalbumin	CAGGUACAG	··· UUUCUAUUCAGU	45 46
Chicken ovalbumin	CCAGUAAGU	··· UUGCUUUACAGG	46
Chicken ovalbumin	AUGGUAAGG	··· CAUUCUUAAAGG	46
Chicken ovalbumin	GAGGUAUAU	··· UGGUUCUCCAGC	46
Chicken ovalbumin	CAGGUAUGG	··· UUUCCUUGCAGC	46
Chicken ovalbumin	AAGGUACCU	··· UUUUAUUUCAGG	47
SV40 late mRNAs SV40 late mRNAs	AAGGUUCGU	··· UUUUAUUUCAGG	48, 49 48, 49
SV40 late mRNAs	CUGGUAAGU	··· UUUUAUUUCAGG	48, 49
SV40 late mRNAs	CUGGUAAGU	··· UUUACUUCUAGG	48, 49
SV40 early mRNAs	AAGGUAAAU	··· GUGUAUUUUAGA	48, 49
SV40 early mRNAs	GAGGUAUUU	··· GUGUAUUUUAGA	48, 49
Polyoma late mRNAs	CAAGUAAGU	··· UAUUUCCCUAGG	*
Polyoma late mRNAs	CAAGUAAGU	··· UUUAAUUCUAGG	*
Polyoma late mRNAs	CAAGUAAGU	··· UCUAUUUUAAGA	*
Silk fibroin	CAGGUGAGU	··· UUUUGUUUCAGU	50
	36 sequences, 26 unique	37 sequences, 31 unique	
Consensus	$^{A}_{C}$AGGUAAGU	UYUYYYU CAGG	

Sequences at splice junctions. The 36 'donor' (5′ end) sequences and 37 'acceptor' (3′ end) sequences represent 43 possible splicing events (each event is depicted on a separate line). Underlined sequences are redundant (either because they are identical to a homologous region or because they represent an alternative splice involving the same region) and were not included in our tabulation. Also not included are sequences at late adenovirus mRNA splice junctions as these are proposed to be recognised by VA RNA[26].

* R. Kamen and B. Griffin, personal communication.

† Refs.

can make at least six (usually eight or nine) of the 11 possible base pairs with U1 RNA.

The complementarity of the U1 sequence to splice junctions in mRNA precursors suggests how hnRNA exons separated by an intervening sequence could be brought into proper orientation for splicing. Because U1 RNA is capable of forming Watson–Crick base pairs with a significant number of residues that lie within the two ends of an intron, a single U1-containing snRNP could interact with both junctions simultaneously (Fig. 4*b*). A similar model has been independently proposed suggesting the involvement of an adenovirus-encoded small RNA known as VA in the processing of late adenovirus messages[26]. Although the complementarity between U1 RNA and splice junction sequences is quite striking, the stability of the predicted helices may be insufficient to achieve the fidelity of splicing observed *in vivo*. Thus, it is attractive to invoke RNA·RNA interactions as only one component of the recognition of intron–exon junctions by a particle containing both U1 RNA and proteins.

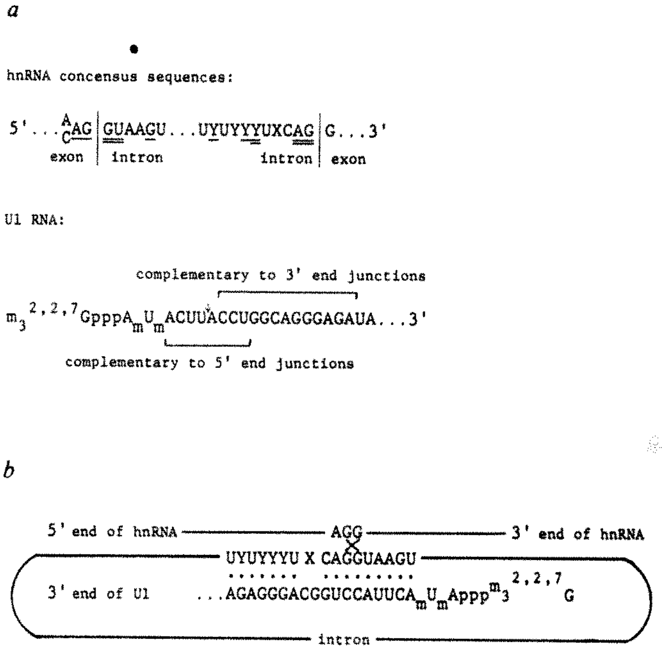
a
hnRNA concensus sequences:
5'...$^{A}_{C}$AG|GUAAGU...UYUYYYUXCAG|G...3'
exon
intron
intron
exon
U1 RNA:
complementary to 3' end junctions
$m_3^{2,2,7}$GpppA$_m$U$_m$ACUUACCUGGCAGGGAGAUA...3'
complementary to 5' end junctions
b
5' end of hnRNA
AGG
3' end of hnRNA
UYUYYYU X CAGGUAAGU
3' end of U1
...AGAGGGACGGUCCAUUCA$_m$U$_m$Appp$^m{}_3{}^{2,2,7}$G
intron

Fig. 4 Possible base-pairing interactions between U1 and splice junction consensus sequences. The U1 sequence is from ref. 7. Sequences used to generate the consensus sequences are presented in Table 1. *a*, To appear in the 5′ or 3′ consensus splice junction sequence, a base must be the most common in that position and occur with a frequency of at least 45%; bases occurring in 75% of the sequences are underlined; those present with 95% or greater frequency are underlined twice. In the position marked $^{A}_{C}$, A occurs 11 times and C 10 times in the 26 unique sequences examined. Y indicates pryimidines. X marks the location of a single nonconserved base in the 3′ consensus sequence. Vertical lines show the most likely location of the splice. The arrow locates the 5′ end of U1a*. All bases occurring with a frequency of 45% or greater are shown. *b*, A possible model for alignment of intron–exon boundaries by base pairing between U1 RNA and sequences at both ends of an intron.

In summary, the abundance of U1 RNA in active cells (about 10^6 per nucleus), the high conservation of both its sequence and the proteins with which it associates, and its considerable complementarity to splice junctions are all consistent with the idea that U1-containing snRNPs have a key role in eukaryotic mRNA processing. They could either be the splicing enzyme itself (note the example of *E. coli* RNase P, which contains a small RNA complexed with several small proteins[27]) or an auxiliary factor required to align splice junctions. One specific prediction of our hypothesis is that avian and insect U1 RNAs should possess 5′ end sequences similar to mammalian U1 as the splice junction sequences determined for the chicken ovalbumin and silkworm fibroin genes show the same degree of complementarity to mammalian U1 as do mammalian splice junction sequences (see Table 1). Likewise, it should be possible to demonstrate direct interaction between a U1-containing snRNP and splice junction sequences in a particular hnRNA. Finally, the introduction of antibodies into living cells or *in vitro* splicing systems[28,29] may result in the inhibition of one or more specific steps in the RNA processing pathway. Experiments relating to this are now underway.

The fact that the other closely related snRNPs (containing U2, U4, U5, or U6 snRNAs) possess common antigenic determinants suggests that they may function comparably to the U1-containing snRNP in RNA processing; different RNA sequences at their 'active sites' could facilitate more precise recognition of variant splice junction sequences in hnRNA or in other RNA molecules. Alternatively, the additional related snRNPs could participate in the transport of RNA molecules from the cell nucleus, as is suggested by the finding that splicing and export seem to be inseparably linked for those hnRNAs that undergo splicing[30,31]. Whatever the outcome, it seems reasonable to investigate further the proposal[26,32–35] that RNA·RNA interactions provide specificity for the precise editing of eukaryotic transcripts; this possibility seems especially attractive when one considers the central role of base pairing interactions in other essential processes (for example, transcription and translation) that developed at an early stage in evolution.

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Plasmid-mediated tissue invasiveness in *Yersinia enterocolitica*

Don L. Zink†¶, James C. Feeley*, Joy G. Wells*, Carl Vanderzant‡, John C. Vickery§, William D. Roof§ & Gerard A. O'Donovan§

*Bureau of Epidemiology, Center for Disease Control, Atlanta, Georgia 30333
† Department of Veterinary Microbiology and Parasitology, ‡Department of Animal Science and §Department of Biochemistry and Biophysics, Texas A & M University, College Station, Texas 77843

Plasmids have an important role in the pathogenicity of certain bacterial species, and *Escherichia coli* provides the most complete example of the relationship involved. Enterotoxigenic strains of *E. coli*, in addition to producing heat-stable and/or heat-labile enterotoxins, may also produce a haemolysin and fimbriate cell surface antigens which facilitate the adherence of the bacterial cell to the mucosa of the small bowel. Numerous studies have shown that these properties are plasmid-mediated[1–5] and that the plasmids act in concert to confer on the host bacterium the ability to produce enteric disease in man and in animals. Moreover, studies with invasive strains of *E. coli* have shown that the Col V plasmid, which codes for the synthesis of colicin V, significantly enhances the pathogenicity of its host bacterium[6,7]. Although the relationship between Col V plasmids and virulence is unclear, reports indicate that Col V-containing strains of *E. coli* are better able to survive in the alimentary tract and that colicine V itself inhibits macrophage function[7,8]. It is probable that bacterial virulence is a complex phenomenon involving both chromosomal and plasmid genes. We describe here a virulence plasmid which mediates tissue invasiveness in human pathogenic strains of *Yersinia enterocolitica*.

Y. enterocolitica is an ubiquitous organism found primarily in the alimentary tract of warm-blooded animals, including man, and in freshwater lakes and streams[9]. Organisms currently regarded as *Y. enterocolitica* are biochemically and serologically heterogeneous[9,10]. Nevertheless, certain biochemical and serological types of the organism are consistently associated with acute gastroenteritis and mesenteric lymphadenitis in man[9]. *Y. enterocolitica* serotypes 0:3 and 0:9 are the most common cause of human infection in Europe, Japan and Canada, whereas serotype 0:8 strains are responsible for infections in the United States[9]. Most strains of *Y. enterocolitica* associated with human infection produce a heat-stable enterotoxin (ST) and demonstrate tissue invasiveness as determined by penetration of cultured mammalian cells or by invasion of the conjunctival epithelium of guinea pigs (Serény test)[11–14,19,20].

We examined the role of plasmids in the virulence of a group of serotype 0:8 strains of *Y. enterocolitica*, obtained during an epidemiological investigation of an outbreak of enteric disease involving 218 school children in New York who had consumed contaminated chocolate milk[15]. The group was interesting because it represents epidemiologically related tissue-invasive and non-tissue invasive strains that are otherwise serologically and biochemically identical. We also examined strains isolated from the incriminated chocolate milk, asymptomatic persons, dairy cattle and water collected from a dairy farm. Those from the latter three sources either did not belong to serotype 0:8 and were believed to be unrelated to the disease outbreak: they were included for comparison with the human-pathogenic serotype 0:8 strains. Each strain was examined for heat-labile toxin (LT) and heat-stable toxin (ST) production, tissue invasiveness (TI) and plasmid DNA content.

A summary of the results of this examination as well as the source of each strain is given in Table 1. No strains produced LT

¶ To whom correspondence should be addressed.

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whereas ST production was common. Only serotype 0:8 strains could invade tissue. Serotype 0:8 strains contained either a 41×10^6 or both, whereas other strains either did not contain plasmid DNA or they contained plasmids with other molecular weights (Fig. 1). These results suggest that tissue invasiveness is related to the 41×10^6-MW plasmid, because all invasive strains contain the 41×10^6-MW plasmids; in addition, the 41×10^6-MW plasmid is not found in non-invasive strains. There was no apparent relationship between plasmid DNA and ST production. Because most strains of *Y. enterocolitica* produce ST, it is possible that ST production is encoded by chromosomal genes[19,20].

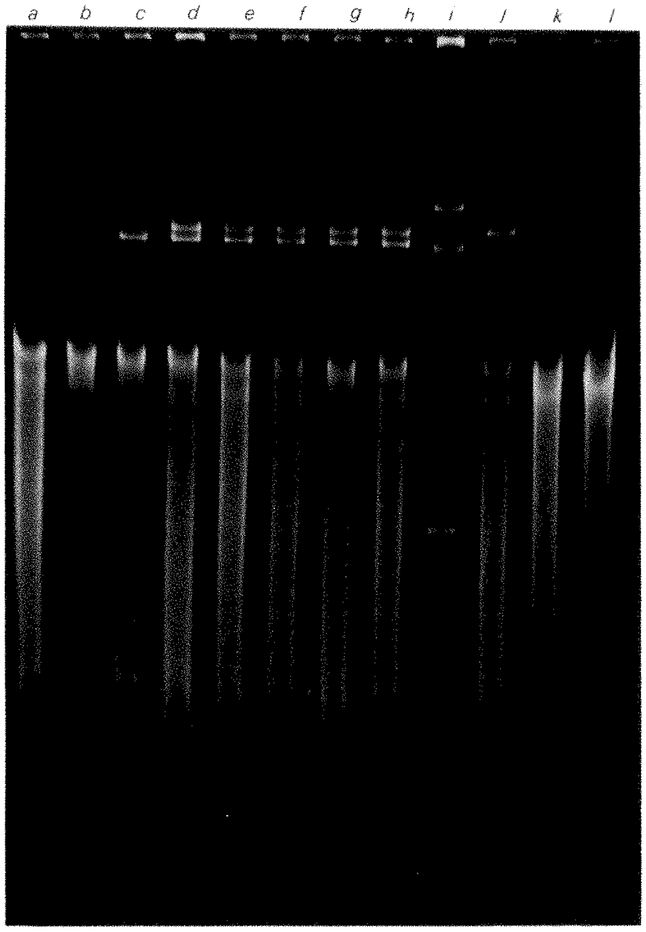

Fig. 1 Identification of plasmid DNA in strains of *Y. enterocolitica*. Partially purified plasmid DNA was isolated from strains of *Y. enterocolitica* and subjected to electrophoresis in 0.7% agarose according to the procedure of Meyers *et al.*[24]. Electrophoresis was carried out at 120 V (31 mA) for 3 h through a 15 cm × 9.6 cm × 0.6 cm gel. After electrophoresis, the gel was stained for 30 min by immersion in electrophoresis buffer (89 mM Tris base, 2.5 mM Na_2 EDTA, 89 mM boric acid) containing 1 μg ethidium bromide per ml. After staining, the gel was rinsed for 1 h under gently running tapwater. The gel was observed and photographed through an orange filter with illumination from a shortwave UV transilluminator. Tracks are as follows: *a*, strain 62, 18 μl partially purified DNA; *b*, strain 61, 18 μl partially purified DNA; *c*, strain 60, 12 μl partially purified DNA; *d*, strain 59, 12 μl partially purified DNA; *e*, strain 58, 12 μl partially purified DNA; *f*, strain 57, 12 μl partially purified DNA; *g*, strain 56, 12 μl partially purified DNA; *h*, strain 55, 12 μl partially purified DNA; *i*, purified standard plasmid DNA (10 μl): upper band, R6-5 (MW 65×10^6); second band, RP4 (MW 34×10^6); third band, S-a (MW 23×10^6); fourth band, pMB9-trimer (MW 10.5×10^6); fifth band, pSC101 (MW 5.8×10^6); sixth band, ColEl (MW 4.2×10^6); *j*, strain 54, 12 μl partially purified DNA; *k*, strain 53, 18 μl partially purified DNA; *l*, strain 63, 18 μl partially purified DNA.

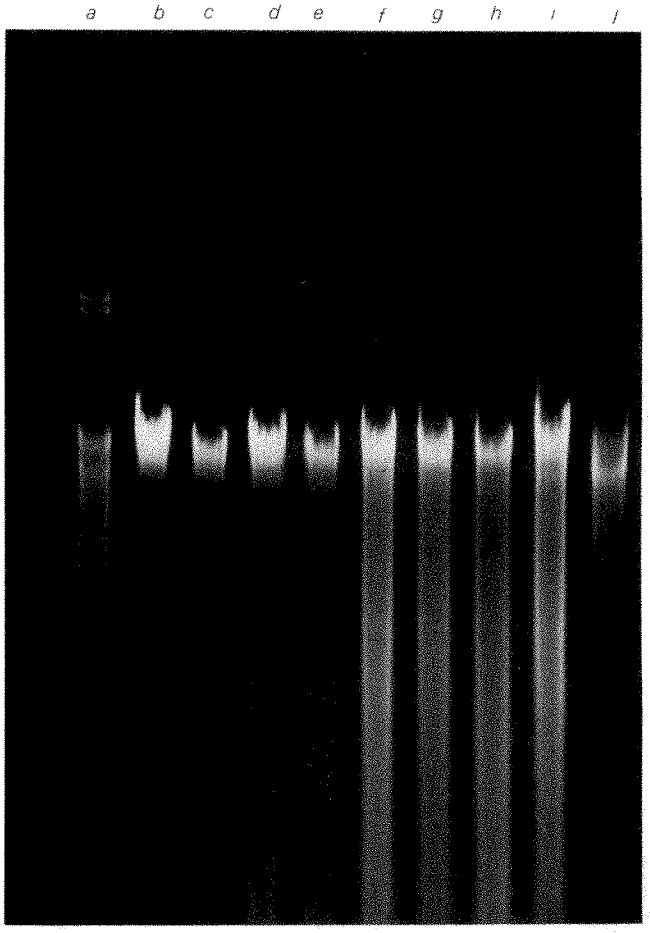

Fig. 2 Agarose electrophoresis of DNA from ethidium bromide-cured strains derived from strain 54. Partially purified plasmid DNA was prepared and electrophoresed as described in Fig. 1. Tracks are as follows: *a*, strain 58, 12 μl partially purified DNA; *b–i*, 25 μl partially purified DNA from eight independently isolated strains derived from strain 54 by curing with ethidium bromide at 35 °C; *j*, strain 60, 12 μl partially purified DNA.

In an effort to confirm the relationship between tissue invasiveness and the 41×10^6-MW plasmid, we grew strains 54 and 58 at 35 °C in the presence of the DNA-intercalating dye, ethidium bromide, which is known to inhibit the replication of some plasmids[21]. Single colony isolates, which had arisen from bacteria grown in the presence of ethidium bromide, were tested for ST production and the presence of plasmid DNA. Isolates derived from strain 54 after curing with ethidium bromide no longer contained plasmid DNA (Fig. 2) and were no longer tissue-invasive. Isolates derived from strain 58 after curing with ethidium bromide now contained only the 35×10^6-MW plasmid (Fig. 3) and were no longer tissue-invasive. Even after repeated subculture, the cured strains did not regain the ability to invade tissue. The 41×10^6-MW plasmid could not be detected in the cured strains by either agarose gel electrophoresis or ethidium bromide-caesium chloride buoyant density ultracentrifugation. We found that the 41×10^6-MW plasmid could be cured from strains 54 and 58 simply by growing them at 35 °C. Table 2 summarises the results of these curing experiments. From these data, it is clear that the 41×10^6-MW plasmid is efficiently cured simply by growing the host strain at 35 °C. These experiments confirm a relationship between tissue invasiveness and the 41×10^6-MW plasmid because loss of the plasmid was always accompanied by loss of tissue invasiveness, regardless of the curing procedure. These results explain earlier observations that some strains of *Y. enterocolitica* lose the ability to invade tissue when grown at 35 °C (refs 11, 14).

When 41×10^6-MW plasmid-cured derivatives of strains 54 and 58 were tested for the ability to produce ST, those treated at 35 °C with ethidium bromide had become temporarily ST negative, while 35 °C alone had not affected ST activity. ST negative strains usually regained ST activity after repeated subculturing.

This further supports the hypothesis that ST production in *Y. enterocolitica* is encoded by chromosomal genes.•

One of the serotype 0:8 strains, strain 60, was isolated from a symptomatic child, yet lacked both the plasmid of MW 41×10^6 and the ability to invade tissue, possibly because this strain was subcultured at 37 °C before inclusion in our study. The other strains used were not subcultured above 26 °C. Strain 54, a tissue-invasive isolate from the incriminated chocolate milk contains only the plasmid of MW 41×10^6. Presumably, the contaminated chocolate milk could have contained as many as four different types of serotype 0:8 *Y. enterocolitica*: those containing no plasmid DNA, those containing both the 35×10^6-MW and 41×10^6-MW plasmids, and those containing either of the two plasmids. We believe that a strain containing both plasmids was responsible for the disease outbreak, because such strains were isolated independently from the faeces of several symptomatic individuals. At this time, it is not known if the plasmid of MW 35×10^6 has a role in human pathogenicity, however, the plasmid of MW 41×10^6 is clearly associated with tissue invasiveness as determined by the Serény test.

We believe this is the first report of a plasmid that mediates tissue invasiveness in a member of the Enterobacteriaceae. These plasmids may also be present in other enteric pathogens, notably *E. coli* because *Y. enterocolitica* and *E. coli* are known to exchange plasmid DNA[22,23]. Furthermore, this report serves to illustrate the value of a molecular and genetic characterisation of plasmid DNA in bacteria isolated during an epidemiological investigation.

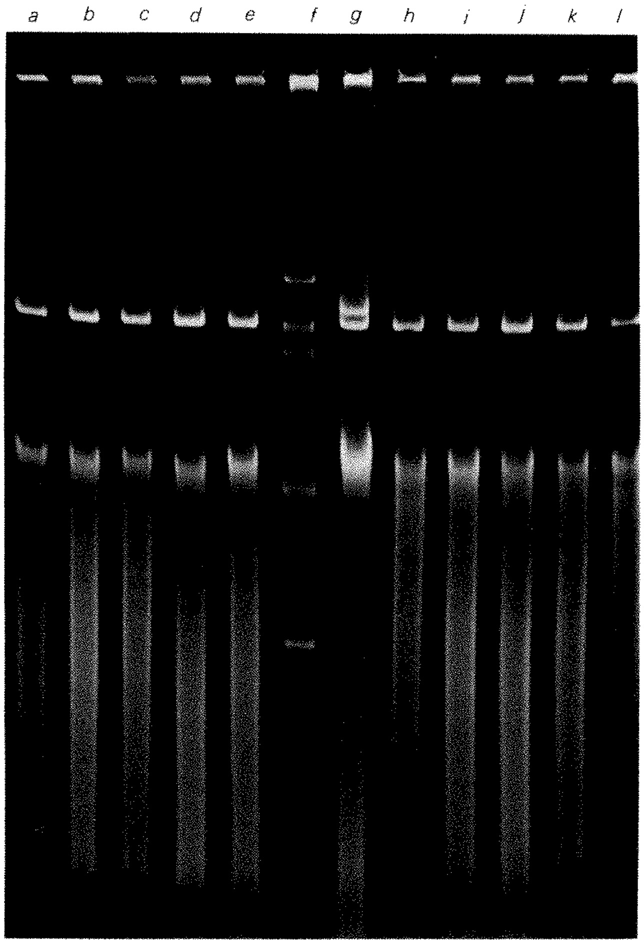

Fig. 3 Agarose electrophoresis of DNA from strains derived from strain 58 by curing with ethidium bromide. Partially purified plasmid DNA was prepared and electrophoresed as described in Fig. 1. Tracks are as follows: *a–e* and *h–l*, 25 µl partially purified DNA from 10 independently isolated strains derived from strain 58 by curing with ethidium bromide at 35 °C; *f*, purified standard plasmid DNA (10 µl) as described in Fig. 1; *g*, strain 58, 25 µl partially purified DNA.

Table 1 Characteristics of strains of *Y. enterocolitica*

Strain no.	Source	Serotype	LT*	ST†	TI‡	Plasmid DNA MW ($\times 10^6$)
62	Cow	4, 33	–	+	–	—
61	Asymptomatic human	5	–	+	–	3.1; 47
60	Symptomatic human	8	–	+	–	35
59	Symptomatic human	8	–	+	+	35; 41
58	Symptomatic human	8	–	+	+	35; 41
57	Symptomatic human	8	–	+	+	35; 41
56	Symptomatic human	8	–	+	+	35; 41
55	Symptomatic human	8	–	+	+	35; 41
54	Chocolate milk	8	–	+	+	41
53	Asymptomatic human	5	–	–	–	—
63	Water	6, 31	–	–	–	—

LT, ST and TI were determined on cultures grown at 26 °C for 48 h (broth cultures were in tryptic soy broth (Difco) with 0.6% yeast extract and agar cultures were on tryptic soy agar, (Difco)).

* Using the Y 1 adrenal cell method of Sack and Sack[16].

† Using suckling mice as described by Dean *et al.*[17].

‡ As determined by the Serény test[18] using a saline suspension (10^{10}–10^{11} cells per ml) of a 48-h agar culture grown at 26 °C. Approximately 20 µl of this suspension was inoculated into the conjunctival sac.

Table 2 Curing the 41×10^6-MW plasmid from strains of *Y. enterocolitica*

	TAMU 54			TAMU 58		
	26 °C	35 °C	35 °C+EB	26 °C	35 °C	35 °C+EB
No. cured†/no. tested	0/16	20/20	22/22	0/18	10/10	8/8
No. TI‡/no. tested	16/16	0/20	0/22	18/18	0/10	0/8

* Ethidium bromide (EB), 15 µg ml^{-1} in Penassay broth, *p*H 7.6, 24 h.

† Strains were examined for loss of the 41×10^6-MW plasmid by agarose gel electrophoresis.

‡ Tissue invasiveness (TI) as determined by the Serény test[18].

We have designated the temperature-sensitive 41×10^6-MW plasmid of strain 54, pDZ19, and the 35×10^6-MW plasmid of strain 60, pDZ20. A physical and genetic characterisation of these plasmids will be published elsewhere.

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Functional and molecular organisation of an antigen-specific suppressor factor from a T-cell hybridoma

Masaru Taniguchi & Izumi Takei

Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan

Tomio Tada

Department of Immunology, Faculty of Medicine, University of Tokyo, Japan

Thymus-dependent (T) lymphocytes have been shown to have antigen specificity. The antigen receptor on T lymphocytes, in contrast to that on B lymphocytes, does not appear to be of the conventional immunoglobulin (Ig) type. Studies on the antigen-specific factors derived from helper and suppressor T cells (Ts) demonstrated that they possess determinants with antigen binding affinity and products of genes in the H-2 complex (MHC)[1–6]. Furthermore, antibodies against the variable region of Ig heavy chains or idiotypes have been shown to react with T-cell antigen receptors as well as antigen-specific helper and suppressor T-cell factors (TsF)[7–15]. It is, therefore, conceivable that at least two gene products are involved in the structural entity of these receptors: one each coded for by genes in either. To establish the molecular nature of the recognition component of T cells we have used homogeneous TsF from a T-cell hybridoma with a specific function. We report here that the antigen binding and I-J coded molecules on TsF are independently synthesised in the cytoplasm, and are secreted as an associated form of the two molecules; this association is required for antigen-specific suppression of antibody response.

Table 1 Presence of the associated and non-associated forms of antigen-binding and I-J coded molecules in the extract and lack of the non-associated form in ascites

Materials added to the culture	Anti-DNP IgG PFC per culture Ascites	Extract
None	1,774 ± 182	1,211 ± 269
Unabsorbed	313 ± 75	146 ± 113
Absorbed with anti-Igs	462 ± 164	131 ± 193
Eluted from anti-Igs	1,802 ± 440	1,547 ± 172
Absorbed with KLH	2,236 ± 174	1,255 ± 465
Eluted from KLH	668 ± 237	219 ± 170
Absorbed with anti-I-J^b	2,043 ± 258	1,342 ± 21
Eluted from anti-I-J^b	580 ± 128	131 ± 193
Combination of materials absorbed with KLH and anti-I-J^b	2,118 ± 335	100 ± 94

The secreted or extracted materials were obtained from suppressor T-cell hybridoma as described previously[16]. Briefly, the hybrids were made by fusion of AKR thymoma cell line BW5147 with the enriched suppressor T cell of C57BL/6 mice specific for KLH. For hybridisation, 5×10^6 enriched suppressor T cells of C57BL/6 origin and 5–50×10^6 BW5147 cells were washed twice in serum-free Dulbecco's modified Eagle's medium (DMEM) and pelleted together at 400 *g*. Polyethylene glycol (2 ml: PEG, molecular weight 2,000)–dimethyl sulphoxide (DMSO) solution (1 weight of PEG plus 1 volume of 15% DMSO–DMEM) was added to the cell pellet and mixed gently. A further 2 ml of 50% (W/V) PEG solution without DMSO was poured into the test tube and the mixture was gently stirred with a Pasteur pipette. The cell suspension was then gradually diluted with 16 ml of serum free DMEM and further diluted with 180 ml of DMEM containing 13% fetal calf serum (FCS). It was then incubated at 37 °C for 3–4 h in 5% CO_2. After incubation cells were washed four times with DMEM and cultured for 1–2 weeks in HAT medium (RPMI 1640 supplemented with 10% FCS and hypoxanthine, aminopterin, thymidine (HAT)) on Falcon plates (Falcon 3008).Cells grown in HAT medium were collected and stained with anti-I-J^b anti-serum (B10.A(5R) anti-B10.A(3R)) and fluorescein-conjugated rabbit anti-mouse Igs. The stained cells were then sorted by fluorescence-activated cell sorter, FACS II (Becton-Dickinson), and I-J positive cells were cloned in a multiwell microplate (Falcon 3040) by limiting dilution or single cell manipulation. So far we have established several I-J positive suppressor T-cell hybridomas with specific functions, that is, cell lines 34S-704, 34S-18, 34S-11, 1L-1, 8C-23, and 9F-18, each of which has the same characteristics as the cell line 9F181a described previously[16]. The cell-free extract used was obtained by ultracentrifugation of the frozen and thawed materials of the hybrid cells (34S-704) at 20,000*g* for 1 h, and the secreted materials were obtained in ascites of hybridoma (34S-704)-bearing F_1 mice. 20 μl of ascites or extracted materials from 3×10^5 hybridoma cells were passed through the immunoadsorbents composed of KLH (5 mg per ml of beads), anti-I-J^b (B10.A(5R)anti-B10.A(3R); 1 ml γ-globulin fraction of antiserum per ml of beads), rabbit anti-mouse Igs (5 mg purified anti-mouse Ig antibodies per ml of beads). The beads used were Sepharose 4B prepared as described previously[16]. The materials absorbed by columns were eluted with 0.175M glycine–HCl buffer *p*H 3.2 followed by an instant neutralisation with 1 M sodium bicarbonate, dialysed for 4–6 h with 0.01 M sodium phosphate-buffered saline *p*H 7.2 and concentrated by negative pressure. The effluents and the eluates from the columns, and the mixture of the effluents from each column were added to the culture of 4×10^6 spleen cells of C57BL/6 mice primed with 100 μg DNP–KLH plus 1×10^9 pertussis vaccine in the Mishell-Dutton system (Falcon 3008 plate), in 1 ml RPMI 1640 enriched with 10% FCS in the presence of 0.1 μg DNP–KLH and 2.0×10^{-5}M 2-mercaptoethanol. Anti-DNP IgM and IgG PFC were assayed on day 5 of culture by the method of Cunningham and Szenberg as described previously[3]. Results are expressed as mean numbers of anti-DNP IgG PFC of four cultures ±s.d.

The T-cell hybridomas with specific suppressive activity were established 20 months previously by the fusion of BW5147 (H-2^k) and enriched Ts of C57BL/6(H-2^b) mice primed with keyhole limpet haemocyanin (KLH). The hybrids continuously secrete specific suppressor molecules with an antigen binding site and products of genes in the I-J subregion of MHC. The properties and functions of the hybridoma-derived TsF have been reported previously[16]. We attempted to separate the active moiety so as to characterise further the structure of the hybridoma-derived TsF. The suppressive extract or ascitic material from the hybridoma (34S–704) was passed through immunoadsorbent columns of KLH, anti-I-J^b and anti-mouse Igs under the same conditions as described previously[16]. The absorbed materials were eluted from columns with glycine–HCl buffer, *p*H 3.2 at 0 °C. Both effluents and eluates were tested for their suppressive activity. As shown in Table 1, the activity of TsF from the hybridoma was absorbed by KLH and anti-I-J^b columns but not by the anti-Igs. The activity was successfully recovered in the acid eluate from KLH and anti-I-J^b columns. These results indicate that TsF in the extracted and secreted materials has both antigen-binding sites and I-J determinants, and can be recovered from either the KLH or anti-I-J^b column. These two determinants are also demonstrated on the surface of semipurified Ts[15,17] and hybridomas[18]. In contrast, recent reports[9,10] showed that isolated T-cell receptor molecules do not contain detectable MHC gene products. It is, therefore, of interest to determine whether or not the I-J determinant is in fact associated with antigen-receptor of Ts, and if such association is indeed required for suppression. If these two distinct determinants are present on a single molecule, the mixture of the materials after absorption with the KLH or anti-I-J^b column should not exert any suppressive activity. On the other hand, if they are on separate molecules and combine when they act on target cells, the mixture should reconstitute the suppressor activity. The mixture of the effluents of ascites from the KLH and anti-I-J^b columns gave no suppressive effect (Table 1). Unlike ascites, the mixture of the effluents of the extracted material definitely produced a strong suppression, despite the fact that either effluent alone had no detectable activity. These

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results indicate that the extract, but not ascites, contains two discrete molecules which would combine to reconstitute suppressor activity. Associated forms of suppressor molecule, however, should be present in the extract, since the activity could be eluted from either KLH or anti-I–J^b column. The secreted material, however, contains only molecules of the associated form.

These experiments do not, however, exclude the possibility that the suppressor activity in the mixture of the two effluents is caused by the additive effect of residual TsF remaining after absorption, even though the effluents alone did not exert detectable suppressor effects. To eliminate this possibility, the extract was passed through the KLH or anti-I–J^b column (first column), and the effluent from each of the columns was successively applied to both KLH and anti-I–J^b columns (second column) (Fig. 1). The effluent from either the KLH or anti-I–J^b column was tested directly for its suppressive activity. In other experiments, the effluent from the KLH column was reabsorbed with another KLH column to ascertain complete absorption. Similar double absorption tests were performed with anti-I–J^b column. The effluents, each after absorption twice with the KLH or anti-I–J^b column were admixed and tested for the suppressor activity. As shown in Table 2, the suppressive activity in the extract was abrogated by absorption with the KLH or anti-I–J^b column (group III or IV). However, the mixture of the KLH→KLH effluent and anti-I–J^b→anti-I–J^b effluent (group V) showed strong suppressor activity. Effluent from the KLH double columns should not contain KLH-binding molecules while I–J molecules are still present. Similarly, after absorption twice with the anti-I–J^b column, I–J molecules are completely removed leaving KLH-binding molecules in the effluent. Hence, the combination of these two effluents resulted in the mixture of the two molecules which reconstituted the suppressive activity. On the contrary, no suppressive activity was recovered when the effluent from the KLH (or anti-I–J^b) column was successively absorbed with the anti-I–J^b (or KLH) column (the mixture of KLH→anti-I–J^b effluent and anti-I–J^b→KLH effluent; group VI). These results suggest that antigen-binding and I–J coded molecules are present independently in the extract, and the association of these two molecules, either of which has no detectable activity by itself, reconstitutes the suppressive activity. Although we have not excluded the possibility that the non-associated form in the extract is due to an inbalance in the synthesis of the two molecules in hybridoma cells, it is concluded that there are at least three molecules in the extracted material: antigen-binding molecule, I–J coded molecule and the associated form of the two molecules. The secreted material consists of the associated form alone. It is, therefore, likely that the two independent molecules in the extract are synthesised in the cytoplasm as a precursor of the suppressor factor which is then secreted after the association.

Our previous studies showed that KLH–TsF specifically suppresses the response of histocompatible spleen cells but not that of allogeneic cells[19]. This genetic restriction is governed by genes in the I–J subregion. The hybridoma-derived TsF also showed rigid genetic restriction[16]. In agreement with this antigen specificity and H-2 restriction of TsF, our present results show firm evidence that the functional structure of TsF is composed of two distinct molecules; one involved in the specific antigen-binding molecule, probably having V_H structure and the other the I–J coded molecule which may serve as a restricting element in the suppressor cell interaction. A definitive answer must await chemical and physiochemical analysis of the hybridoma-derived suppressor molecule.

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Table 2 Requirement of the co-existence of I–J bearing and antigen-binding molecules for the manifestation of suppressor function

Experimental group	Materials after absorption with: first column	→ second column		Anti-DNP IgG PFC per culture
I	None			2,824 ± 98
II	Unfractionated			525 ± 196
III	KLH	None		1,722 ± 224
IV	Anti-I–J^b	None		2,923 ± 598
V	KLH	→ KLH	combined	383 ± 150
	Anti-I–J^b	→ Anti-I–J^b		
VI	KLH	→ Anti-I–J^b	combined	2,507 ± 287
	Anti-I–J^b	→ KLH		

The extracted materials (34S-704) unabsorbed (group II), absorbed with the KLH (group III) or anti-I–J^b (group IV) column, or the mixture of the effluents from the double columns (groups V or VI) as indicated in Fig. 1 were added to the culture of DNP–KLH primed spleen cells from C57BL/6 mice and tested for their suppressive activity. The assay of anti-DNP IgG PFC is the same as in Table 1. Results are expressed as mean numbers of anti-DNP IgG PFC of four cultures ±s.d.

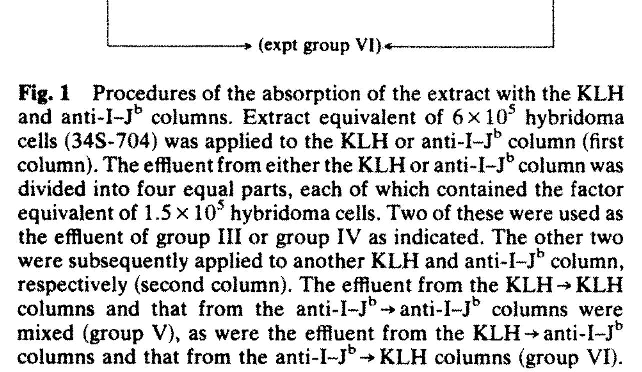

Fig. 1 Procedures of the absorption of the extract with the KLH and anti-I–J^b columns. Extract equivalent of 6×10^5 hybridoma cells (34S-704) was applied to the KLH or anti-I–J^b column (first column). The effluent from either the KLH or anti-I–J^b column was divided into four equal parts, each of which contained the factor equivalent of 1.5×10^5 hybridoma cells. Two of these were used as the effluent of group III or group IV as indicated. The other two were subsequently applied to another KLH and anti-I–J^b column, respectively (second column). The effluent from the KLH→KLH columns and that from the anti-I–J^b→anti-I–J^b columns were mixed (group V), as were the effluent from the KLH→anti-I–J^b columns and that from the anti-I–J^b→KLH columns (group VI). The unabsorbed (group II), the absorbed (groups III and IV) or the mixture of the effluents absorbed with double columns (groups V and VI) were added to the culture and their suppressive activities were tested in comparison to the response of group I without the factor. The materials of each group should contain the factor equivalent of 3×10^5 hybridoma cells.

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Dioxin as a health hazard

Alastair Hay

VIETNAM and Sev́eso are the two principal subjects in this book on dioxin contamination. Vietnam had about 100 kg of 2, 3, 7, 8 — tetrachlorodibenzo-p-dioxin (dioxin) dumped on it in the course of the US military defoliation programme over the country from 1962-1970. Sev́eso, a small Italian town some fifteen miles north of Milan, was also the hapless victim of dioxin when in July, 1976, a reactor manufacturing trichlorophenol overheated. The reactor contents, including some 1-1.5 kg of dioxin, were vented into the atmosphere and precipitated out over the town and its environs.

The author of this book quite clearly chose Vietnam and Sev́eso to make the point so aptly summarised in the title. It is Whiteside's thesis that contamination of the environment by dioxin poses a considerable health hazard, and that time to deal with it may be running out.

Much of the material in this book is not new and was first published as two long articles in the *New Yorker.* Whiteside is a staff writer for the magazine and his book is written in the same relaxed style which is a characteristic of this American weekly. The result is that the message is hammered home, all the harder; Whiteside is out to convince readers of the dangers posed by dioxin.

The narrative begins with Vietnam and the military defoliation programme during which over 11.0×10^6 gallons of the herbicide Agent Orange (a 1:1 mixture of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2,4-dichlorophenoxyacetic acid) was sprayed on forests and food crops. This, however, is history. What concerns Whiteside is the fact that 2,4,5-T is still used in the United States. It is 2,4,5-T which contains the dioxin contaminant, carried over from an earlier step in the manufacturing process when trichlorophenol is produced. And dioxin is now known to be a teratogen, mutagen and carcinogen.

The Pendulum and the Toxic Cloud: The Course of Dioxin Contamination. By Thomas Whiteside. Pp. 205. (Yale University Press: New Haven, Connecticutt, and London, UK, 1979.) £10.80.

Lessons learned in Vietnam about the havoc caused by the herbicides are used by the author to illustrate the risks entailed by the continued use of 2,4,5-T. There are parallels between 2,4,5-T use in Vietnam and in the US, but there is one important difference. The concentration of dioxin present in 2,4,5-T in the US is probably about 1/100th of that used in Vietnam. Curent formulations of 2,4,5-T contain less than 0.1 p.p.m. of dioxin. There are those who argue, however, that even this concentration is high enough to present a health risk. Whiteside takes the same view.

The US Environmental Protection Agency (EPA) is also concerned about 2,4,5-T and in March this year issued a banning order restricting its use. According to the EPA there is a correlation between 2,4,5-T spraying of forests in the state of Oregon and an increase in the rate of spontaneous abortions in the area. The EPA announcement came too late for inclusion in Whiteside's book, nevertheless, the author has captured the changing mood in the US about the wisdom of using 2,4,5-T. In an enlightening anecdote, he reports that "The attitude toward 2,4,5-T has somewhat changed at the Department of Defense it seems. The days are over when during the height of herbicidal operations in Vietnam, an American military spokesman could declare that the relevant herbicidal agents were "not at all harmful to humans or animals" — and illustrate this statement by dabbing on his tongue a bit of liquid from a bottle that sat on his desk".

Sev́eso is a familiar enough story to readers of *Nature.* When the reactor in the ICMESA chemical plant at Sev́eso overheated discharging its toxic contents over the surrounding countryside, it caused one of the most serious pollution incidents yet recorded. It also caught the Italian authorities unprepared. Whiteside cites the illogicality of some of the early restrictions on movement of people into and out of the most seriously contaminated zone (Zone A), at Sev́eso. The authorities, according to Whiteside, "First forbade all entry into it [Zone A] by outsiders and then changed the rules to allow ten persons to enter on weekdays and twenty on Sundays". "When people in the area heard about these safety measures, some of them just laughed, in spite of the tragedy they were caught up in," one person familiar with the evacuation told me. "They would say to one another, 'so dioxin is twice as dangerous on weekdays as it is on Sundays?'."

Although the Italian authorities perhaps deserved the censure they have received, it should not be forgotten that it was a reactor owned by Givaudan — a Swiss multinational and subsidiary of the giant pharmaceutical company F. Hoffmann La Roche — that caused the accident. Givaudan have always insisted that their reactor design was adequate to meet with foreseeable accidents. Perhaps Sev́eso was unforeseeable. But it happened, and the reactor design did not meet the situation.

The author does not deal in great detail with the reactor design, preferring instead to concentrate on the consequences of its failure. Whiteside has interviewed many of the scientists and politicians involved with the aftermath of Sev́eso. Few of them stand out as luminaries. Many of them revealed their party political interest — a loyalty which inhibited the work of the committees concerned with the decontamination and health surveillance programme.

Much of Whiteside's story is concerned with the residents of Sev́eso. Many lost their livelihood; most received compensation for this loss, but the bitterness still lingers and it comes through in this book.

There are a few points which could give the account a slightly different balance. The author has on several occasions been too selective in his choice of material. For example, there are three studies which now show 2,3,7,8-tetrachlorodibenzo-p-dioxin to be carinogenic. Two of these agree on the dose level at which dioxin exhibits its carcinogenic effect, the third predicts the chemical to be a carcinogen at dose levels several orders of magnitude lower. Whiteside only publicises the latter. Then there is the case of the link in several Swedish hospitals between hexachlorophene (a bacteriacide made from trichlorophenol) use and birth malformations. The author retells this story, but omits to mention that it has not been confirmed, and that the Swedish Health Authority is sceptical about the link.

There is an error in the description of the effect of the 1968 explosion in the trichlorophenol reactor at the UK company, Coalite and Chemical Products Ltd. Whiteside says several workers died after the accident as a result of severe liver damage. This is not the case. Only one worker died, and this was as a direct result of injuries received when the reactor blew up. In Europe, however, workers formerly employed on trichlorophenol reactors have died, but so far there is no firm evidence to link these deaths with dioxin exposure.

Apart from these criticisms the book makes good reading. It ought to be read by everyone concerned with how relations between the general public and government authorities can go wrong. But the picture is neither as black, nor as clear cut as the author makes out. There are many questions about dioxin still to be answered. □

Alastair Hay is Lecturer in the Department of Chemical Pathology at the University of Leeds, UK.

Hippocampal function

T.V.P. Bliss

The Hippocampus as a Cognitive Map. By J. O'Keefe and L. Nadel. (Clarendon/Oxford University Press: Oxford, 1978.) £25.

THE hippocampus, a phylogenetically primitive cortical structure long admired by neuroanatomists for the simplicity and regularity of its neural architecture, came to more general notice in the 1950s with the discovery that a bilateral lesion of the hippocampus in man causes severe impairment of memory. This glimpse of the engram aroused great excitement and for the past two decades rats with hippocampal lesions have been among the commoner experimental species in behavioural laboratories around the world. Disappointingly, the benefits of all this effort have not been startlingly apparent. What differences in behaviour can be found between normal and hippocampal rats cannot easily be explained in terms of simple loss of memory. Alternative concepts, such as inhibition and frustration, emerged as candidates for a theory of hippocampal function, but to no very general acclaim. For a time there was a vogue for Keating's hypothesis which allocated to the hippocampus the task of preventing the neocortex from falling into the lateral ventricle and drowning. Meanwhile, and particularly in the past decade, workers from the other end of the neurobiological spectrum — anatomists, physiologists and pharmacologists — have found in the hippocampus a uniquely convenient model for studying synaptic organisation and function in the mammalian cortex, with the result that there is today probably more information, from more sources, about the hippocampus than any other structure in the mammalian brain.

It is an appropriate time therefore for a new look at the problem of hippocampal function. What is needed is a theory which tells us not only what the hippocampus does but also how it does it. This is what O'Keefe and Nadel set out to achieve in their ambitious and stimulating book. According to their theory, the hippocampus is the chartroom of the brain's navigational system, the repository where neural representations of an animal's environment are assembled and stored. The theory derives from the discovery by O'Keefe and Dostrovsky in 1971, that there exist 'place' neurones in the rat's hippocampus which fire only when the animal is at a particular place in the environment; and from a reassessment of the behavioural literature which allows the authors to conclude that it is only in situations where an animal has to identify a place, that hippocampal animals show deficits in behaviour.

The book opens with a discussion of post-Newtonian ideas about space and continues with an illuminating analysis of the ways in which an animal can find its way around space. O'Keefe and Nadel distinguish between two possible navigational strategies (route hypotheses and place hypotheses) based on the two kinds of space which are represented in the brain: relative space, which is centred on the observer, so that spatial relationships between objects in the environment change as the observer moves; and absolute space, which is non-egocentric and items within which are fixed relative to each other. Absolute space can be represented by a map, the neural representation of which, in O'Keefe and Nadel's theory, is located in the hippocampus.

The second section of the book is concerned with the anatomy and physiology of the hippocampus, and opens with a comprehensive account of the afferent and efferent connections of its two principle cell populations, the granule cells of the dentate gyrus and the pyramidal cells of the hippocampus proper. The rather more selective coverage of hippocampal physiology includes a discussion of the properties of place units and a good account of the mysterious theta waves which can be recorded in the hippocampus of conscious animals during voluntary behaviour such as active exploration. The mechanism responsible for generating theta activity is not well understood, though there is some evidence to support O'Keefe and Nadel's assumption that it plays a role in synchronising the excitability of cell populations in the hippocampus.

The authors then present a model of how the hippocampus might function as a map-generating device. Information about the environment is passed to the granule cells from entorhinal cortex. Each granule cell receives a number of entorhinal fibres, at least two of which, representing some specific combination of sensory stimuli, must be active to fire the cell. The output from each granule cell is fed via synapses *en passage* to a large number of pyramidal cells, whose excitability is modulated by theta waves propagating across the hippocampus as the animal moves through its environment. At a given moment therefore only a proportion of the pyramidal cells receiving impulses from an active granule cell will be at the appropriate phase of theta activity to fire. The set of activated pyramidal cells, each one responding to a particular combination of features in the environment, constitutes the neural representation of place. In order to account for the fact that place units continue to fire if any small subset of the set of stimuli which define the environment is removed, it is assumed that the set of active pyramidal cells become functionally linked together through the strengthening of synaptic connections between them. In the third stage of the model a population of pyramidal cells compares the expected with the perceived environment and fires whenever there is a mismatch, thus providing the trigger for renewed exploration. Although the model makes a valiant attempt to exploit the peculiar characteristics of hippocampal anatomy and physiology, including theta activity and long-term potentiation of synaptic connections, it is nevertheless open to many objections. Much of its anatomical substrate is speculative at best, and in the several hippocampal pathways in which the phenomenon has been demonstrated, long-term synaptic potentiation seems to be a function of activity in afferent fibres alone, rather than of conjoint pre- and post-synaptic activity, as the model requires. A more fundamental objection, and one which the authors don't discuss, is how an animal recognises a familiar place when it is approached from a different direction.

In the third and longest section of the book, O'Keefe and Nadel turn their attention to the behavioural literature, and in particular to the learning ability of rats with hippocampal lesions. Their industry here is overwhelming — there is seemingly nothing which they have not read, tabulated, critically assessed. The various types of behavioural experiment are carefully explained and for the reader without experience of the psychological literature this is an outstandingly good introduction. The conclusion the authors reach at the end of this exhaustive survey is that, in general, and as the theory would predict, behavioural deficits in hippocampal animals only emerge in tasks which the intact animal solves using place hypotheses; even then the hippocampal animal may be able to compensate by using alternative route-type strategies. This distinction between the use of place hypotheses in normal animals and the compensatory use of route hypotheses in hippocampal animals brings a considerable degree of order to a literature rich in apparently contradictory results. Nevertheless, doubts remain. During the course of their review a barrage of analytical ingenuity is directed against experiments which do not at first sight fit the prediction of the theory. The effect of this is ultimately to raise doubts in the reader's mind about the ability of any experiment, whatever its results, to furnish convincing evidence against the theory.

The final section is taken up with a discussion of the amnesia which results from hippocampal lesions in humans. This is a controversial area with no general agreement about the extent to which amnesia is a failure of recall rather than a failure of storage. O'Keefe and Nadel propose that the human hippocampus is reserved for a context-dependent memory system, having a spatial component equivalent to a cognitive map, which is located in the right hippocampus and a verbal component responsible for memory of connected discourse or narrative, which is represented in the form of a semantic map in the left hippocampus. They conclude that while the use of strategies designed to assist recall can often improve the performance of amnesics in context-free tasks, such as memorising word lists, this is not the case for context-dependent memories: here the loss of memory is total and irrecoverable.

Both authors were at one time students of D.O. Hebb, and their book, though it lacks the stylistic charm of Hebb's monograph *Organization of Behaviour*, follows the same tradition in its attempt to fuse neurobiological and behavioural data into a unified theory of function. The verdict on the theory must, at the moment, be 'unproven'. But whether or not the theory survives, O'Keefe and Nadel have in this book provided a solid basis on which the question of hippocampal function can, at last, be sensibly debated; on that account alone their book is a very considerable achievement. □

T.V.P. Bliss is a member of the Division of Neurophysiology and Neuropharmacology at the National Institute for Medical Research, London, UK.

Nuclear and reactor physics concepts

Malcolm C. Scott

A Guidebook to Nuclear Reactors. By A.V. Nero. (University of California Press: Berkeley, Los Angeles and London, 1979.) Hardback £17.50; paperback £5.95.

ALMOST without exception, nuclear power programmes throughout the world have been subjected to critical, and often hostile, scrutiny. There seems to be no single reason for these examinations; certainly, it is not related to the actual safety record of nuclear power reactors, as most re-appraisals predate the accident at the Three Mile Island pressurised water reactor plant at Harrisburg, Pennsylvania. Rather, the opposition to nuclear power has centered on a number of issues. The first of these is the relationship between nuclear power and the spread of nuclear weapons; the second relates to the problem of radioactive waste disposal and the associated question of whether or not we know enough about the effects of radiation; and the third is concerned not with nuclear power *per se*, but with the role of high technology in society.

To an interested but uninformed spectator some of the confrontations which have occurred must have seemed baffling. However, the worst aspect of this is possibly not the fact that 'experts' frequently hold diametrically opposite views but, rather, the fact that, as it *is* high technology, the whole field of nuclear power and radioactivity is relatively inaccessible. The few books written for the layman are too simplistic to allow an understanding of the issues involved (inasmuch as they are technical rather than sociological or political), whereas the specialist monographs are incomprehensible, assuming a background which the layman does not have. Even for professionals, much of the information required to counter opposition arguments is scattered through the literature, and there has been no single book giving the necessary background technical information across the whole nuclear front.

A Guidebook to Nuclear Reactors was written specifically to try and overcome these problems. It is intended for both the non-technically trained and the professional needing a wide ranging but concise guide to the whole nuclear power field.

The book is divided into four parts. The first, entitled "A General Introduction to Nuclear Reactors", starts by outlining the main features of a nuclear power plant and then goes on to consider the effect which such a system has on its surroundings, in terms of both heat and radioactivity discharges. At the end of this section there is a chapter on accidents, discussing how they can arise and what the consequences may be, and concluding with an introduction to risk assessment along the lines of the American Rasmussen Study.

In the second part there are four chapters, each dealing with a specific reactor type, namely pressurised water reactors, boiling water reactors, heavy water reactors and gas-cooled thermal reactors. Here each chapter has the same format, namely a description of the layout and principal components of each reactor type, followed by a description of auxiliary systems. Next comes a discussion of the safety systems incorporated in the reactor, and there is then consideration of fuel utilisation and reactor operation.

The third section, also of four chapters, starts off by considering the question of uranium resources and then goes on to discuss the rôle of both fast breeder reactors and high conversion efficiency thermal systems. The various nuclear fuel cycles are then outlined in the context of fuel processing, which leads naturally to a chapter on "The Weapons Connection". Finally, in the fourth section, there are three chapters on advanced reactor systems ("Breeders, Near-Breeders and What Not"), the last chapter including a mention of fusion, fission-fusion and accelerator-based nuclear fuel breeding systems.

Let it be said immediately that this is an extremely good book. However, the concentration on reactor systems found in, or of interest to, the United States means that systems in, for example, the USSR, together with our own in Britain, get scant mention ("In addition, England has constructed gas-cooled reactors with carbon dioxide coolant"). There is some repetition, which may have a didactic purpose, and some Americanese ("The style of the book is more informational than argumentative"), though mercifully little.

Nevertheless, faced with the abundance of extremely useful tables and figures, it is churlish to emphasise these faults. For the professional involved in nuclear debates and for the student entering the field this is an invaluable book. However, its value for the layman is likely to be limited by the sheer quantity of information and density of technical terms which, even when explained, need to be absorbed in order to appreciate the text. Nevertheless, as a source of background information for the non-scientist it remains the best book which I have seen, and Anthony Nero deserves credit for trying to raise the level of the so-called 'nuclear debate'. □

Malcolm C. Scott is a Senior Lecturer in the Department of Physics at the University of Birmingham, UK, in charge of the MSc course on the Physics and Technology of Nuclear Reactors.

Transport organ physiology

O.H. Petersen

Membrane Transport in Biology. Vol.IV A and B. Edited by G. Giebisch, D.C. Tosteson and H.H. Ussing. Pp.471; 939. (Springer: Berlin, Heidelberg and New York, 1979.) Two volumes DM296; $162.80.

RECENT progress in the study of transport has been astonishing. This is clear when reviewing the impressive series on *Membrane Transport in Biology,* which has now been brought to an end with volume IV (consisting of two books) dealing with the transport organs. The editor of this last volume, Gerhard Giebisch (Yale), is to be congratulated on providing a number of good chapters written by scientists who are among the leaders in their fields of study. It was also a good idea to have asked Renkin (California) to provide an introductory chapter on transport across capillary membranes. However, while there is no denying the general high quality of the individual contributions, this volume is marred by an unfortunate lack of balance. Of the 18 chapters, no less than 10 deal exclusively with the kidney. In contrast, there are only two chapters on the intestine. Salivary glands, gastric secretion, exocrine pancreas, gall bladder and liver cells have one chapter each. This is clearly not a reasonable balance either in terms of current research interests or with respect to general importance. Furthermore, it means disturbingly uneven presentation with regard to depth of treatment and level of detail. Thus, while Boulpaep (Yale) devotes 47 pages exclusively to a discussion of kidney electrophysiology, Schultz (Houston) uses 31 pages in the only chapter on the small intestine and Schulz and Ullrich (Frankfurt) have 41 pages at their disposal to deal with all aspects of pancreatic membrane transport biology, including the electrophysiology! Nevertheless, Schulz and Ullrich's chapter on the pancreas is a model of clarity and also seems more up to date than many others, betraying the fact that the greater part of these two books must have been finished in late 1976 or early 1977, although some more recent references seem to have been brought in at the proof stage.

The many chapters on kidney transport biology have their problems. In spite of separate chapters on electrochemistry (Khuri, Beirut) and electrophysiology, there is extensive repetition, particularly of the electrophysiology (even of some of the figures!) in chapters on sodium chloride (Windhager, Cornell) and potassium transport (Giebisch, Yale). One figure illustrating Na-glucose co-transport in membrane vesicles (data of Kinne, Frankfurt) is shown three times (pages 156, 426 and 555). The interaction between transport of sodium and organic solutes, originally discovered by Weymouth Reid at the turn of the century, rightly features prominently both in the kidney section, in an excellent review by Ullrich (Frankfurt), and in sections on the intestine and the pancreas.

Immediately following the massive kidney sections is a comprehensive chapter on the salivary glands by Young (Sydney). It is perhaps surprising that salivary glands should be much more extensively treated (more than 100 pages) than the stomach (Forte and Machen, California) or the pancreas, but at least we have here a very complete (up to 1977) account of the transport physiology of these glands. The completeness is, however, somewhat at the expense of clarity. It seems unlikely, for example, that the non-specialist will find the 20 pages dealing with salivary gland electrophysiology (not a single illustration here!) easy going.

One cannot help wondering what readership the editor of this beautifully produced volume had in mind. The 10 very detailed kidney chapters must be attractive to nephrologists who might also enjoy the comparatively short but generally clear accounts of the other transport organs (or some of them; where is, for example, the placenta?). The title is, however, misleading, as it seems to announce a comprehensive treatment of membrane transport in the transport organs. What a pity that this last volume has only become a *Membrane Transport Biology* for nephrologists! □

O.H. Petersen is Professor of Physiology at the University of Dundee, UK.

Autoradiographic techniques

Tim Appleton

Techniques of Autoradiography. Third edition. By A.W. Rogers. Pp.429. (Elsevier/North-Holland: Amsterdam, New York and Oxford, 1979.) $59; Dfl.133.

IT is always a difficult thing to write a book covering a wide range of techniques. Rogers has tackled his task with an infectious enthusiasm and I admire the courage and hard work which has gone into this work. Unlike the earlier editions, this edition is conveniently split into three main parts; the theoretical basis of autoradiography (129 pages); the planning and interpretation of autoradiographic experiments (151 pages); and description of autoradiographic techniques (126 pages). There is also a useful appendix of data which any autoradiographer will appreciate.

I wish I could be enthusiastic about this book. As with earlier editions the descriptions of techniques are mingled in with the text. It would be much more helpful to the beginner and expert alike if detailed descriptions of techniques were set out clearly in the form of a schedule, in heavy type-face, so that they can be easily found and followed. The chapter on photomicrography is not as helpful as it could be, because the quality of the illustrations is very poor — worse than in the first edition. I suspect that this may be the fault of the publisher, as some of the figures which are 'identical' with those in the first edition are clearly at a higher magnification in the third edition, yet the captions still show the lower magnification. Figure 54 in the third edition is of the same field as figure 31 of the first edition; both figures show a magnification of × 135, yet the third edition plates are magnified a further 1.4 times. Such errors are frequent and should have been noticed by the author at the proof stage. Considerable clarity has been lost in many of the illustrations and some of the line drawings are so faint in the review copy that they became irritating. Captions are frequently placed in awkward positions and orientations.

References throughout were fairly complete but did not give titles. Occasionally I found that the reference quoted was not always the authoritative one but taken from a symposium report or review. This resulted in some inaccuracy which might confuse the reader.

The first edition of this book appeared in 1967, was 335 pages long with 77 illustrations. The third, "completely revised", edition has grown to 429 pages with 98 illustrations. I thought that the first edition was too long and my immediate reaction to the latest edition is much the same. However, there is often some benefit in discussing techniques in such a lengthy manner; I found my interests in autoradiography rekindled as I read on. Such length is only really acceptable if the price can be kept at a reasonable level, and I fear that a cost of around £30 will prove too high for the younger scientist.

It is inevitable that a book can never be up to date, and this is certainly true of this one. I would have liked to have seen more discussion of the use of scintillators to increase autoradiographic sensitivity, and on the localisation of diffusable substances at the level of the electron microscope. But then the book would have been even longer and, I fear, more expensive still. □

Tim Appleton is Lecturer in Physiology at the University of Cambridge, UK.

nature

17 January 1980

A new responsibility for science

A major impact of public reaction to the Soviet invasion of Afghanistan has been to legitimate the slowing down — and in some cases even the reversal — of efforts to limit the global spread of nuclear weapons. The most obvious manifestation of this process has been President Carter's decision to defer consideration by the US Senate of the new Strategic Arms Limitation Treaty (SALT II), concluded last year after lengthy negotiations with the USSR. But the impact of the Soviet moves is already making itself felt across a wide range of non-proliferation initiatives — and the net result can only be to bring the threat of nuclear war a step nearer.

It seems inevitable, for example, that the new strains on East-West relations will provide one more hurdle to the successful negotiation of a comprehensive test ban, severely decreasing hopes for an early agreement. In Pakistan, US moves to cut off military and economic aid last year in an attempt to wean the country away from nuclear weapons production are now being reversed to help provide a counterweight to the Soviet presence in nearby Afghanistan. And to mollify Pakistan's own neighbour, India, State Department officials in Washington are said to be dropping their opposition to the export of highly-enriched nuclear fuels for the Tarapur nuclear plant, despite India's continued refusal to accept international safeguards.

To warn of the dangers inherent in these moves is in no way to condone the Soviet Union's actions, nor to deny that the USSR must share a large part of the blame for their implications. But as far as US policy is concerned, it is important that the realities of the situation are not confused with images of that reality.

The paradox of President Carter's position, for example, is that by taking a firm stand on international issues such as Iran and Afghanistan, he has regained a reputation as a political leader previously lost for failures in relatively unrelated domestic spheres, namely energy and rising inflation. Furthermore the defense industry had not been slow to point out the domestic merits of increased military spending. Pentagon officials, for example, point out that in the past defense-sponsored research has provided a major boost to civilian technologies — and that given current national concerns about declining rates of innovation, increasing the military R&D budget could again help efforts in this direction.

The volatility of US politics tends to create a rhetoric designed primarily for domestic consumption. And in many ways the potential political gains that might have been achieved by supporting SALT have been overtaken by the gains of undercutting the same agreement.

No-one can pretend that the way forward for arms control is clear. But one strategy that could be pursued with vigour would be to separate discussions aimed at limiting nuclear weapons as far as possible from the temporarily sinking ship of *détente*. In the past, the scientific community has ably taken on this role, using its position on the fringes of the political establishment to keep open lines of communication that might otherwise be lost. Last week the council of the American Association for the Advancement of Science passed a resolution emphasising the continued need to support arms control efforts, and drawing attention to the particular responsibility of scientists to help this process (see page 234). We support the AAAS's efforts, and urge British scientists to do likewise. □

No engineers without teachers

AT last the Finniston report on the training and status of engineers in Britain has been officially published. The history of leaks of the report has been so long that it is rather an anticlimax to have the real thing on one's desk*; *Nature* described the bulk of its recommendations last summer (2 August 1979, page 352). We support most of them as indicating a practical, as opposed to big-headedly political route towards the improvement of Britain's economic competitiveness. Britain has too long neglected and rejected the applied scientist and engineer as a creator of wealth; the paradigm of economic activity has been the City, where paper rather than materials are moved: a second-order economic activity. There is a strong element of class-consciousness in this anti-technical valuation, and it pervades the whole of the establishment, including the Civil Service. Even the Department of Industry is neglectful of technological change.

Perhaps Finniston can blow a wind of change through all this — but it is unlikely. A sense of depression is increased if one looks at the figures for the numbers of science teachers in training in England and Wales — for these are the people who will influence the outlook of the next generation. Take physics and chemistry, for example. Of 19,500 physics teachers in secondary schools in January 1977, only 43% were actually qualified to teach physics. Of 18,800 chemistry teachers, half were qualified. The majority of teachers were 'filling-in', when their basic training was in another subject. In January 1979, the annual number of new physics teachers needed to fill vacancies and begin to replace unqualified teachers was estimated by the Department of Education and Science to be 1,300. In chemistry the number was 600.

Against this, in 1979 just 244 physics and 345 chemistry graduates entered one-year DipEd courses to learn to teach — satisfying roughly one fifth of the need for physicists and one half that for chemists.

Mathematics is worse and biology better off than this. Of 48,300 maths teachers (maths is a compulsory subject), 62% are qualified, and there is an annual need (DES figures) of 4,300. The input of graduate trainees in 1979 was 636 — one seventh the need. In biology there are 20,900 teachers of whom 55% are qualified; the annual need is 600; and the 1979 graduate input was 657, satisfying demand.

In all subjects the position is worsening. Three years previously there were 412 graduate physics entrants into teacher training (244 in 1979); 438 chemists (345 in 1979) and 800 mathematicians (636 in 1979). (Biology fell slightly from 691 in 1976 to 657 in 1979.)

Without well-qualified science teachers, there is little hope for the future of science and engineering in Britain. Now is also time to discuss how to attract good science teachers. □

*'*Engineering our future'. Report of the Committee of Inquiry into the engineering profession. HMSO, £5.*

Editor of Nature

NATURE is pleased to announce the appointment of Mr John Maddox as Editor. Mr Maddox is presently Director of the Nuffield Foundation and was Editor of *Nature* from 1966 to 1973. He will take up his appointment not later than 30 June. MacMillan Journals are particularly appreciative of *Nature's* development during Dr Davies' editorship and it is Mr Maddox's intention to build on his achievements.

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United States

David Dickson reports from the annual conference of the American Association for the Advancement of Science in San Francisco

Council supports arms control

FOLLOWING a public meeting at which a number of scientists described the increasingly urgent need to limit the proliferation of nuclear weapons, the council of the American Association for the Advancement of Science agreed last week to establish a working group "to help organise and mobilise resources towards nuclear arms control."

The AAAS council also agreed that efforts for directing science towards peace rather than war should be a major theme of next year's annual meeting, which will take place in Toronto.

Both decisions formed part of a resolution on nuclear weapons control submitted by seventeen members of the association, which pointed out that "improving the effectiveness of science in the promotion of human welfare is an objective and a special responsibility of the AAAS."

By adopting the resolution, the AAAS council agreed to support both US efforts to obtain effective bilateral nuclear arms limitations and the completion of the Comprehensive Test Ban Treaty. It also expressed opposition to "the development by any country of new weapons systems which make verification more difficult, or pose a first-strike threat."

Supporters of the resolution point out that the latter is an implicit reference to, among other things, the recent decision by President Carter to recommend the deployment of a new generation of Minuteman missile, the MX.

The council also agreed to support the development of plans for the step-by-step conversion by all nuclear-weapons-producing nations of facilities for nuclear weapons production, research and testing, into science and technology facilities for peaceful uses. Such "conversion" plans are expected to be made the subject of a special session at next year's meeting of the association. □

Gene resource programme called for

In a further resolution, the AAAS council gave its support to efforts to develop a national gene resource conservation programme. A resolution was passed which argued that there is insufficient support in the US for addressing the rapid depletion of germplasm resources of natural ecosystems, and that there is currently no comprehensive or effective national programme which would ensure that important germplasm resources are safeguarded.

Military support of basic research defended

RECENT increases in military support for basic research are rekindling the fierce debate over its social and ethical implications dormant for much of the past decade following widespread criticism during the Vietnam war era.

Supporters of military funding for science, addressing a session of the AAAS meeting, not only emphasised its importance for the long-term development of new weapons systems, but also claimed that through technological spin-offs — which have ranged from synthetic rubber to large-scale integrated circuits — military-supported research can help stimulate general industrial innovation in the US.

Critics, however, argued that the military control of research meant such research would inevitably be skewed towards the production of weapons of mass destruction, and was therefore in fundamental conflict with the well-being of mankind.

Dr George Gamota, director for research in the office of the Under-Secretary of Defense for Research and Engineering, told the audience that the department was trying hard to repair the working relationship with the scientific and engineering communities that had been weakened in the mid-1960s and early 1970s.

Particular emphasis was being paid to restoring defense support for university research. Forty per cent of the basic research currently funded by DoD was carried out by universities; and over the past three years, university support increased by nearly 70%, although overall support for basic research increased by only 30%.

The need for military support for basic science, said Dr Gamota, "is rendered even more urgent by the relative decline in the extent of basic research conducted internally by industry or supported by the industrial sector on the academic community."

Referring to the Mansfield Amendment, passed in 1971 and restricting DoD support to research with a potential relationship to military needs, Dr Gamota said there was nothing in the act which touched upon the department's ability to support basic research. "Only the availability of funding and the level of interest of other agencies determines how wide a spectrum of research support is possible in the context of the DoD mission," he said.

Dr Gamota argued that it was impossible to single out any major basic research effort, particulary a new field, that could be categorically ruled out as potentially irrelevant to the Defense Department. "Our job is to follow the national trend and ensure that adequate funding is provided in areas of potential interest to DoD, and primarily only funding limitations and the extent of other agency interests limit our scope". The aim and goal of the department's support of basic research was "to ensure that the support will keep us technologically ahead of the world."

A full-blooded attack on such thinking, however, came from Professor George Wald, emeritus professor of biology at Harvard University, who accused military planners of helping create a "mad nightmare of a world in which we are putting our children."

In contrast to those who argued that the US was now lagging behind the USSR in military technology, Professor Wald said that America was still five years ahead, both qualititatively and quantiatatively. And although he admitted that he had no confidence in the peaceful intentions of the Soviet Union, it was the US, he said, which was currently leading the arms race.

Earlier in the conference, Professor Philip Morrison of the Massachusetts Institute of Technology, argued that through a mixture of judiciously chosen policies it should be possible to cut the military budget by 40% to 50% while preserving the same level of national security as exists at present.

"The key point is to buy what we need, and organise to use it as we need. We need not buy systems because one or another service or firm wants us to do so, or because once in another world they were useful, or because the Soviet or someone else has them," Professor Morrison said.

Recent events at the global and domestic level made it important that scientists in the AAAS and elsewhere take a close look at science in the service of war. "The US has largely paced the arms race," he said. "We can cut military research and development to a reasonable size, and restore the talent and resources thus set free to the growing needs of civil society." □

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Nuclear accident casts a long shadow

EVIDENCE that last March's accident at the Three Mile Island nuclear power plant has already cast a long shadow over public attitudes towards a broad range of activities assosicated with nuclear power was presented by various speakers to last week's meeting of the American Association for the Advancement of Science.

Speaking at a session on risk in technological society, Dr Peterson, until recently director of the US Congress' Office of Technology Assessment and a member of the public commission established by President Carter to report on the accident, said that in the climate following the accident, public reaction to any incident involving a major release of radioactivity into the environment would almost certainly lead to demands for an end to the use of nuclear energy. "If in the interim we have tied our way of life to electricity from nuclear, the economic and social costs will be major indeed," he warned.

Dr Peterson drew attention to recent recommendations that plans for evacuating the residents close to a nuclear plant be developed before a plant is authorised to operate. Several nuclear physicists with the Nuclear Regulatory Commission, for example, had recently said that in the event of a future nuclear plant accident — and given the same amount of information as was available at the TMI incident — they would again recommend evacuation.

"Together these facts demonstrate that when individuals have the opportunity to dig deeply into the operation of nuclear power plants, they conclude that it is only a matter of time before an accident releasing a devastating amount of radioactive material occurs. Why else would we need evacuation plans?" he asked.

Dr Peterson suggested that although the potential for a catastrophic accident raised the most amount of public concern, a more serious risk in the long run could come from the cumulative impact of countless minor releases of radioactivity. These ranged from uranium mill tailings, through the disposal of radioactive wastes, to the use of part-time employees as "sponges" to carry out brief tasks in highly contaminated areas to save regular employees from excessive exposure.

Another lesson discovered recently, he said, was that the nuclear industry had not learnt its lesson. He quoted, for example, advertisements that had been placed in major newspapers by the Edison Electric Institute, claming that the electric companies agreed with the Kemeny Commission's message that nuclear power could proceed, but with caution.

"The Kemeny Commission said no such thing," protested Dr Peterson. "The introduction to our report carefully explains that our assignment limited us to matters bearing directly on the Three Mile Island accident, and that we made no judgements on a number of other issues — for example, the disposal of radioactive wastes — that must be considered in deciding whether nuclear power is basically safe."

A different slant on the impact of increased public concern about nuclear hazards was given by Nobel prizewinner Dr Rosalyn Yalow in the course of a public lecture on radioimmunoassay.

Dr Yalow referred in particular to the recent press coverage that accompanied the decision by various states temporarily to close radioactive waste disposal sites. She suggested that the so-called problem of radioactive waste disposal from hospitals and medical centres was really not a problem but rather a figment of the "small minds and fertile imaginations" of various regulatory agencies.

"An unreasonable fear of any level of radiation associated with radioactivity or nuclear energy generated by newspaper headlines and publicity seekers was now pervasive throughout our society," Dr Yalow said. "Because of this we dissipate research talent and funding on make-work projects in response to unreasonable fears in make-work situations."

She cited, for example, the recent announcement that a number of research groups are to study possible long-range health effects secondary to the reactor accident at Three Mile Island. The maximum estimated dose received by anyone living near the reactor during the accident was bout 70 millirems, less than one third the amount they would have received by spending a year in Colorado.

"If these were the maximal doses received one can predict with absolute certainty that, other than psychological effects, no physical aberration would be demonstrable. Yet we waste time, money and research talent — and generate more psychological stress than would otherwise occur in unnecessary make-work of no scientific merit." □

Women scientists still lose out to men

DESPITE almost a decade of affirmative action and raised consciousness, women continue to face a number of barriers in pursuing a scientific career, according to Mrs Betty Vetter, executive director of the Scientific Manpower Commission.

Speaking at a session on employment opportunities for science and engineering doctorates, Mrs Vetter said that women had approximately doubled their share of doctorates in science and engineering since 1970. But they still tended to be employed in lower-paid jobs than men, to have more difficulty in gaining tenure at universities, and to suffer a much higher rate of unemployment.

"It is not at all uncommon to find persons who believe that affirmative action programmes for women or for minorities have given women an edge in employment opportunities, but the statistics do not seem to bear out an assumption of equal treatment, even at the entry level," she said.

For example, while it might be expected that women who obtained their PhDs in the 1950s and 1960s had not advanced as rapidly as men from the same cohorts, one might expect to find that those from more recent classes would be advancing at rates comparable to men: yet evidence produced by a survey carried out last year by the National Academy of Sciences showed that this was not true.

In the life sciences, for example, 23% of academically-employed men earning PhDs in the period 1970-74 had been appointed assistant professor by 1977, but only 11% of women in academic careers earning PhDs in the same period. In contrast, 27.5% of the women PhDs held the rank of lecturer or instructor, compared to 15.5% of the men.

In the federal government, a number of studies had shown that women still tend to advance in their careers more slowly than men, overall lagging by an average of 1½ to 2 civil service grades. For example, in 1978 women made up almost 31% of all microbiologists employed by the federal government, but their median grade — and their salary average — was about 20% less than that of their male counterparts.

The best possibility for reducing inequity of opportunity for young women research workers appeared to be in industry. In 1977, for example, only 10.8% of all women doctoral scientists and engineers were employed by industry.

Evidence that the general employment prospects for young research scientists and engineers is likely to remain gloomy through most of the 1980s was presented by Mr Alan Fechter, head of scientific and technical personnel studies for the National Science Foundation.

This situation was created by a number of factors, such as the increased hiring of research workers into non-tenure-track positions, a general reduction of promotion opportunities, declining salaries relative to other sectors, and cutbacks in 2-year and marginal 4-year institutions as a result of falling student enrolments. □

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Study shows only 1% real growth in basic research spending

THE combined effects of increasing inflation and Congressional budget-paring have meant that a strong push by the Carter administration for continued real growth in funding for basic research was successful "to only a limited degree" over the past year, according to a survey of actions taken on the 1980 budget prepared by the AAAS secretariat and published last week.

The survey points out that, as in previous years, Congress substantially increased the basic research budget of the National Institutes of Health but pared back the increases proposed for most other agencies.

"Excluding NIH, the total income increase in basic research over the fiscal year 1979 comes out at about 11%, compared to 13% proposed in the budget. With inflation approaching 10%, compared to less than 7% anticipated in the budget, not much real growth is left."

The survey points out that although the administration failed to convince Congress to hold R & D funding for the NIH to the level appropriated for 1979, the rate of increase was substantially slowed from that enjoyed by NIH in recent years.

In contrast, although Congress had initially moved to reduce the proposed levels of expenditure for military research and development, as attention focused on SALT II and on Iran, legislators changed course and approved virtually the full amount recommended by the President.

"In general, Congress seems to be fully committed to providing full support to a large and expanding military R & D programme" says the survey. It points out that no major defence policy issues were fought out between Congress and the administration in the discussion of the military R & D budget.

The net effect of congressional actions, it concludes, was to raise support for basic research by one per cent. □

Carter plans 13% research boost

PRESIDENT Carter is to ask Congress for an increase of about 13% in financial support for basic science in the fiscal year 1981 — just enough to keep slightly ahead of expected inflation and to allow for real growth.

In his budget request, due to be presented to Congress on 28 January, the President is also expected to place particular emphasis on funding for engineering and the physical sciences, which have recently received less attention than the biomedical sciences.

The President gave a preview of his budget request on Monday at the presentation of 20 national medals of science, the highest honour awarded to US scientists and engineers by the federal government. He said that the net effect of his request will be to increase support for basic science by 40% during the first three years of his administration — officials said later that the increase for the first two years was 24%.

The budget for the National Aeronautics and Space Adminstration is expected to reflect the impact of the additional costs of the space shuttle on space programmes. In particular, NASA officials have been unable to reverse an earlier decision by the Office of Management and Budget not to support research on an ion drive system which is essential if a joint mission is to be mounted to the comet Tempel 2 with a 'fly-by' of Halley's comet in 1985.

However, this year at least two projects are expected to be approved: the gamma ray observatory, and a national oceanographic satellite.

David Dickson

United Kingdom

Locust research cut

RESEARCH on locusts at the UK Centre for Overseas Pest Research is one of the casualties of recent cuts in government spending. For the past 50 years, the centre has combined research and field control and has been a major centre for visiting scientists from developing countries to do research and for the training young scientists.

With the cuts, all field work, research and training will stop and the locust experts employed by COPR will be available only as consultants. Within six to eight years, according to Dr Haskell, director of the centre, most of them will have left. The United Nation's Food and Agriculture Organisation in Rome has taken over the monitoring of potential areas of plague evolution and of organising international control measures but Dr Haskell fears that much of the centre's basic research on locusts will not be taken up elsewhere.

The centre's first director was Sir Boris Uvarov who determined that what had been thought of as two distinct species of locust were different forms of one species: the less innocuous 'grasshopper' and the plague-causing destroyer of crops. More recently, the centre has been renowned for its work on the relation of swarming to meteorological conditions and in establishing the importance of pheromones to swarm evolution.

Peter Collins

United Kingdom

BIONIC BRITANNIA
WE USED TO HAVE THE TECHNOLOGY

Time running out for technology

FAR from making a smooth transition into the post-industrial world, Britain is heading for much greater competition, says a government report* published last week.

Britain must be prepared particularly for developments in "information technology and biotechnology, and of industries of high growth potential arising from the increasing need to conserve energy and materials" it says.

The booklet is the result of a working party, headed by Sir James Menter, of the UK's Advisory Council for Research and Development. ACARD called for the report principally to project the impact of potential technological change on employment, but the authors found it impossible to reach quantitative conclusions. But they argue "more unemployment results from loss of market share following a failure to innovate than from the introduction of new technology. Conversely, if new technology leads to an increase in market share there is generally an increase in employment opportunities".

The least predictable recommendations in the report are that the UK should consider setting up an agency to facilitate the import of new technologies from countries which have already taken a lead; and that large companies with R & D results that they do not intend to use should be encouraged to set up or seek out small firms better able to utilise such results.

The report suggests that "attempts by firms or industries in the UK to avoid competition from technologically more adept foreign competitors by specialising in certain (usually high-priced) sectors of the market (for example the motor cycle industry), by continuing to accept lower real wage rates and higher manning levels (car industry) are ultimately doomed to fail."

Robert Walgate

**'Technological Change: threats and opportunities for the UK,' Cabinet Office, HMSO, £1.75*

0028-0836/80/03236-01$01.00

Italy

Health monitoring endangered at Séveso

THE lack of facilities available to local scientists for monitoring the health of people exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) at the Italian town of Séveso in July 1976 is endangering the health surveillance programme.

This is just one of the conclusions reported in the minutes of the International Steering Committee (ISC) of the Lombardy region's special office for Séveso. The ISC — composed in the main of internationally renowned scientists — was formed in the wake of the Séveso accident to consider the implications of exposure to dioxin by the local population.

To save the health surveillance programme, the ISC says it is of crucial importance for either local or national government to make a commitment to establish more permanent staffing within an epidemiological group set up specifically for the "Séveso follow-up". And in order to overcome the disillusion of local doctors in the current surveillance exercise, the ISC consider that one of the first priorities of the epidemiological group must be to enhance the motivation and to increase the involvement of local medical practitioners and hospital staff in a long-term programme.

It is the view of the ISC that the skin disease chloracne is one of the best indications of exposure to dioxin. Individuals with chloracne, it says, represent a group "for whom biologically significant (as distinct from presumed) exposure [to dioxin] can be reasonably accepted". The ISC believes that it is this group which faces the greatest health risk and which should be monitored for many years, perhaps even for life. To assess the risks for this sector (a group composed entirely of children) it is suggested that two matching control groups — one not exposed to dioxin, and the other possibly exposed — should also be monitored.

Dioxin is known to be both a teratogen and a carcinogen in animals. Data on the danger it poses to the human fetus are simply not available. However, the ISC acknowledges that there may still be long-term risks to the fetus of "chronic exposure to very small amounts" of dioxin and the committee recommends that a registry be kept of severe and minor congenital malformations; birth weight; prenatal and postnatal mortality rates; and the rate of spontaneous abortions.

An efficient cancer registry based on morbidity as well as mortality is also urgently required says the committee. Maintenance of such a registry is both difficult and time consuming, but the ISC points out that many countries carry out such programmes.

Over 1,000 individuals employed in the chemical industry are known to have been exposed to significant levels of dioxin over the last 30 years and the committee is anxious to obtain any information about their health problems. The ISC says that this information will help the Italian authorities to assess the adequacy of the health programmes for the Séveso area.

Failure to implement these proposals will mean that much information, important to the well being of the Séveso population, will be lost. If the ISC recommendations are ignored, many of the scientists on it could withdraw.

Alastair Hay

Eastern Europe

Scientific exchanges are needed for détente

SCIENTIFIC exchanges "agreed by treaty"should continue, irrespective of developments in Afghanistan, according to East German radio last week.

East Germany has a special interest in preserving *détente* at this stage — the first ever formal summit between Party Leader Erich Honecker and the West German Chancellor Helmut Schmidt is expected this spring. However, political spokespersons and commentators throughout the socialist bloc seem to be making a concerted effort to minimise the effect of the Soviet intervention in Afghanistan on *détente* in Europe.

Eastern hopes in this regard have undoubtedly been bolstered by the slow response of Western European governments in support of Carter's measures. The UK, for example, will hold a Cabinet Office meeting later this week to discuss British action (if any). An environmental working party is currently in Moscow, discussing odour prevention, under the auspices of the Intergovernmental Joint Commission for Trade and Technology. According to a representative of the Department of Trade, the meeting was probably allowed to continue because no member of the British delegation was of sufficiently high rank to warrant its cancellation at this stage.

Accordingly, while denying that the Soviet Union has, in fact, done anything untoward in sending forces into Afghanistan, the socialist bloc commentators continue to urge *détente.* "Economic, scientific, technical and cultural links between countries", said Viktor Glazunev, one of Moscow Radio's chief "news analysts", "make up what you might call the material fabric of *détente.* A blow at the fabric strikes a blow at *détente* itself . . ."

The need to bolster *détente* is all the more urgent, with the Hamburg Scientific Forum only a month away. This forum is intended to be a meeting point for individual scientists to discuss problems in the context of East-West scientific cooperation, including contacts, communication and the exchange of information. Clearly world events, and the general issues of academic freedom and human rights are bound to be raised in that context.

Indeed, one human rights lobby, led by Valentin Turchin, and John Macdonald Q.C., would have suggested that western delegates should press for the forum to be postponed, until such time as the dissident physicist, Yurii Orlov, is released from strict regime labour camp. (The rationale of their argument being that Orlov, convicted in 1978 of slandering the Soviet state, had, in fact, been collecting material on alleged Soviet breaches of the Helsinki accords, in his role as founder member of the clandestine Helsinki Monitoring Group in Moscow.)

Other action groups, while agreeing that the forum should take place, see it as a valuable platform for human rights. "In our opinion", says the Scientific and Medical Committee for Soviet Jewry, "for the exercise to be meaningful, not only should the Hamburg meeting include on its agenda the question of the freedom of the movement of scientists; also any scientific body must ensure that a clause dealing with this problem is written into its constitution."

This too is essentially the attitude taken by Bruce Kiernan, human rights coordinator of the American Association for the Advancement of Science, which, together with the Committee of Concerned Scientists will be holding a human rights' briefing for the US delegates to Hamburg early in February.

Those French scientists that are concerned with human rights have not yet made a formal statement; it is understood, however, that they will press for the guaranteeing of the personal security of scientists as an indispensible preliminary to any international cooperation — and that, the Helsinki accords being indivisible, it is impossible to dissociate "exchange programmes" from human rights.

Vera Rich

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NEWS IN BRIEF

China issues new titles for engineers

CHINA'S State Council has issued temporary provisions for titles for the country's 21.5 million engineers and technicians. Issued on 28 December, the council's recommendations call for the titles, senior engineer, engineer, assistant engineer, senior technician, and experienced worker or technician to be granted according to previous contributions, vocational proficiency, previous schooling and seniority. The senior technician or experienced worker designations will be granted to those who have special skills, can solve key technical problems and have distinguished themselves in work. An editorial in the *Beijing Workers Daily* says the provisions "will help overcome the egalitarianist tendency that calls those who work more and better and those who work less and worse the same". Assessment will be done by special groups consisting mainly of experts. Party committees should guide the groups instead of interfering with their remit, the editorial says.

French unions refuse reactor safety report

FRENCH trade unions representing nuclear reactor workers have refused to accept the conclusions drawn in a safety report issued by the French electricity board. Released to the unions last week by the Director of Equipment, the 43 page report concludes that the cracks found in the tubular heat exchanger plates in the reactors, Tricastin I, Gravelines I and Dampierre I "do not constitute a risk to the workers or the population" and that "all the faults will be able to be monitored during the reactor's operation." The Communist trade union, the CGT, has demanded increased safeguards in the functioning of the safety valves in the primary cooling circuit. The socialist trade union, the CFDT, has objected to "numerous uncertainties in the security and safety of the installations" and the unaligned Force Ouvriere protested a lack of completeness in the report. "The politics of nuclear information is a reality and we cannot tolerate witholding information of any sort".

French Academy elects first woman to full membership

THE French Academy of Sciences has, for the first time in its 300 year history, elected a woman as a full member. She is Mme Yvonne Choquet-Bruhat, a leading figure in the field of general relativity for 30 years, who has pioneered work on the Cauchy problem and the mathematical structure of Einstein's equations. The Academy, which admitted Pierre Curie and closed its doors to Marie Curie, has thus finally, if belatedly, recognised the contributions of women to knowledge. Speaking at the time of her election Mme Choquet-Bruhat said that she hoped she would not be the sole woman member of the Academy for long. Now that the breach has finally been made, it is expected that many more of France's talented women scientists will follow.

Jim Ritter

British nuclear reactors closed for cracks

TWO Magnox gas cooled reacors at Dungeness near Dover, have been closed for an indefinite period because of cracks in the expansion bellows of their primary cooling circuits. The cracks, up to a meter long, are located in the welds of the expansion units inserted into the 4 ft diameter cooling pipes. Originally discovered during the biennial routine inspection of reactor 1 in August 1978, the cracks were ruled to have been present during manufacture and not to have grown during the lifetime of the reactor. But a more detailed ultrasonic search on reactor 2 during its biennial inspection in April 1979, showed meter long cracks in many of the 24 bellows mountings. Reactor 2 is still shut down. The Central Electricity Generating Board, in conjunction with the Nuclear Installations Inspectorate then decided to close down reactor 1 for further tests last weekend. The CEGB will prepare a detailed safety report on the situation which must be passed by the NII before either reactor is restarted. According to the *Guardian*, engineers are reported as saying that the 16 year old reactors are unlikely to ever be used again.

British groups organise against transport of nuclear waste

THE London Regional Anti-Nuclear Alliance has announced a campaign to prevent the weekly shipment of three tons of nuclear waste from Dungeness to the Windscale reprocessing plant from passing through London. Citing US legislation which bans nuclear waste transport through New York City as a precedent, the Alliance says that the three previous derailments of trains carrying radioactive waste means that "it is only a matter of time before a serious accident occurs". According to the Alliance an accident damaging the flasks could cause contamination within a 12 mile radius producing 8000 additional cancers and would render the area within a 3 mile radius uninhabitable for 70 years. The Alliance says that the flasks have not been tested to withstand impacts in excess of 30 miles per hour or fires lasting longer than 30 minutes and it calls for a rerouting of trains around London and other densely populated areas as well as the release of the government's evacuation plans in case of an accident.

Piperno still held without evidence

THE case of Italian physicist Franco Piperno, extradited to Italy from Paris last summer for "actions against the State" has entered a new phase. After 22 months of hearings and 20,000 pages of documents on the assassination of Aldo Moro, seven magistrates have recently published a 186 page report in Rome. The magistrates could find no evidence linking Piperno and his two colleagues in the Workers Autonomy Movement, Antonio Negri and Lanfranco Pace, with the assasination and have called for an additional inquiry. The report says that it "is extremely difficult to furnish proof, and because of the complexity of the accusation against them, the case of the three Autonomy leaders ought to be separated from that of the Red Brigades and a supplementary inquiry be effectuated". According to *Le Monde*, the prosecution believes it may find the evidence it needs from the recent testimony of an informer, Carlo Fioroni.

Bangladesh LANDSAT programme enters second phase

A THREE day international workshop on disaster preparation and remote sensing inaugurated the installation of an advanced ground station in Bangladesh for receiving satellite data on flood indications and major meteorological disturbances. Automatic data collection platforms will also be set up along the major rivers in Bangladesh to assist in water management and planning. Chaired by Dr B.M. Padya, Chief Technical Advisor of the World Meteorological Organisation, the conference was attended by representatives from Bangladesh, India and Thailand.

Satellite data has already been used in Bangladesh for land accretion and land use studies, changes in water reservoir levels and siltation surveys. Remote sensing has revealed desertification in northern districts and LANDSAT information has been used for afforestation projects, crop distribution studies, estimation of crop yields and salinity intrusion studies.

M. Kabir

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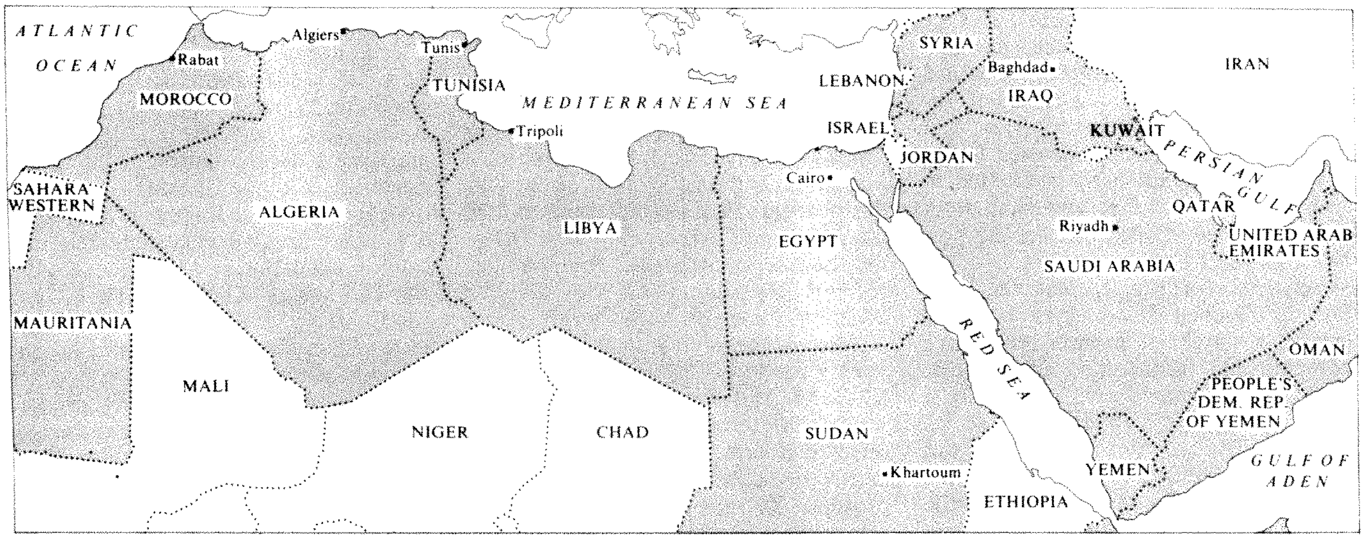
FEATURES



Shaded countries are members of the League of Arab States. Somalia, not on the map, is also a member

Planning science in the Arab world

Many problems faced by scientists in the Arab world are common to all Third World countries. **Dr A B Zahlan** takes the Arab case to test some general assumptions about the problems of science and technology in developing countries

SCIENTIFIC research in the Arab world has been growing at a steady pace during the past three decades. Two thirds of this activity has been in the agricultural and medical fields where extensive institutions and qualified manpower now exist. The research work is generally of an applied nature and dwells on current problems. By contrast, research activity in all other fields is on a limited scale. In the basic sciences,for example, it does not account for more than 5% of publication output, and in areas of a vital developmental, industrial and defence nature — such as metallurgy, civil engineering, hydrocarbon chemistry, electronics, aerospace, — it is on a vary small scale.

The fields of scientific research that are normally undertaken by university professors have been severely affected by the generally poor working conditions in the fifty or more Arab universities. Teaching loads are high, job security is low, libraries are poor or non-existent, laboratory and work-shop facilities are restrictive, administrative red tape is excessive, intellectual stimulation is limited, standards for academic promotion are wanting, and technical and secretarial support staff remain scarce. These conditions of course do not prevail at the same level in all institutions, and an active research programme often flourishes in difficult circumstances thanks to the energies of a few researchers. Moreover, a small number of scientists have created permanent links with research groups in Europe or the US.

Various statistical compilations set a figure of about 40,000 for the number of researchers in Arab countries. These staff more than 500 R&D institutions and 50 universities, the latter enrolling more than 1 million students. However, the constraints on their capabilities have reduced their contributions to the scientific literature to a meagre 1,500 per annum. The absence of adequate publications in the applied sciences at one stroke limits the development of appropriate teaching materials and condemns workers to researching and rediscovering similar problems. The present level of funding in the range of $1-2 per capita per year during the 1970s has, of course, also been a constraining factor.

Despite these drawbacks, the scientific communities in the Arab world have accumulated considerable experience and have experimented with a broad range of ideas and instruments. Many of the problems faced by Arab researchers are common to Third World countries, and the Arab experience thus has a universal value.

The concepts underlying much of current thinking in science and technology in developing countries emphasise the need for high level manpower, the integration of research activities in science and technology with development plans, the central planning and management of the activity, the linking of the central management, (often called 'science policy making body', SPMB) to the highest decision maker, and so on. These notions are so reasonable that very little in the way of empirical testing has been attempted. The Arab experience in the use and application of these notions has made it possible to evaluate these concepts in real-life situations.

One finds, for example, that science policy making bodies still subscribe to the belief that the shortage of scientific manpower is an obstacle to the development of research programmes. However, the expansion of the secondary and university educational systems in the Arab world as well as the access to foreign educational systems (the USSR, eastern and western countries, and North America) have combined to eliminate the supply of high level manpower as the determining factor in scientific research or in any other function where such manpower is needed. Thus, although the Lebanese National Council for Scientific Research (NCSR) subscribed to the principle that the formation of scientific manpower was a necessary step prior to the funding of research projects, only 10% of its own grants went to recipients of NCSR

Dr Zahlan is currently researching the history of technical change in the Arab world. He was professor of physics at the American University in Beirut and is a member of the UN Advisory Committee on Science and Technology. His book Science and Science Policy in the Arab World is published by Croom Helm this week.

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advanced study fellowships; and the recipients of the remaining 90% of the grants had not required NCSR support for their higher education, having completed their advanced education before the NCSR adopted its manpower formation policy.

The feasibility of centrally planned scientific activity has been taken for granted by developing countries, and especially by UNESCO's Science Policy Division. Egypt has been committed to such an approach. More than eight major changes in its SPMB's during the past two decades clearly undermines the effectiveness of central planning. The Science Council (established in 1956) created a system of committees for the planning and management of scientific activity. Between 1958 and 1960, it mobilised 3,000 Egyptian scientists to prepare a five-year plan. This effort was sidetracked, however, in 1961 by the establishment of the Ministry of Scientific Research. There were further changes in 1964, 1965, 1968, 1971, 1975 and finally in 1976.

Since 1956 the councils and committees established to plan and manage scientific activity have grown in size and number. By 1977 the Academy of Scientific Research and Technology had 17 research councils and 97 subject committees involving more than 1,300 members for planning and managing its programmes. Of the 97 subject committees only a third held one or more meetings attended by 50% or more of their committee members; and 53% held no meetings. A detailed analysis revealed that there was no correlation between the number of meetings held and the amount of funds appropriated, or between the declared priorities of the Academy's science policy and the activities of the committees or councils or the funds appropriated. Although a basic objective of the Academy is to concentrate on the solution of urgent problems, only 5.6% of 1977 appropriations were committed to projects whose duration was two years or less, and 51% were granted to projects whose duration was five to ten years.

The linkage of the SPMB to the decision-makers has posed a number of interesting problems. UNESCO, for example, advocates that this linkage be to the highest decision maker in the country in order that financial support be guaranteed and that the integration between development planning and science and technology be efficiently effected. Despite the importance given to this linkage in the international literature on the subject, one finds surprisingly little concern for the actual behaviour of these Third World institutions. Since SPMB's are central to the relationship between the scientific community and the decision maker, their stability is vital to the development of effective personal and institutional relationships. The eight major changes in the SPMB in Egypt noted earlier reduce the credibility of such an assumption; such a high level of discontinuity in the institutional framework undermines both the linkage to the decision maker and the human and social relationships within the community itself. In addition to institutional instabilities one must also consider the frequent changes of the officials responsible for the SPMB.

The situation on the decision-maker's side is no less incoherent. Frequent changes in ministers and in policies also occur. Civil disturbances and economic crises take their toll of scientific activity. The first year of the Lebanese civil war, for example, reduced the output of the scientific community there by more than 42%.

Much of the international discourse on science and technology in the contexts of UNCSAT and the UN conference on science and technology for development revolved around the notion of "applying" science and technology to the development process. Yet the major obstacles to achieving such a useful objective were left out of most of the deliberations. These obstacles are due to the structure of the planning process, the inadequate communications between the scientific community and planners, bureaucratic resistance to data collection and dissemination, and the domination of the central planning process by economic, political and financial considerations. The widespread utilisation of the turnkey type of contracts with foreign firms reduces opportunities for applying indigenous science and technology considerably. The character of government operations is important, rather than the institutional link of the SPMB to central authority. As a matter of fact, it does not seem to matter whether the linkage is to the head of state or to the minister of higher education and scientific research.

Most of the Arab countries have now reached a phase where the major obstacles to further progress lie in the weaknesses of the existing institutions, the planning practices of ministries, the means available to university professors to undertake scientific research, and cultural policies.

The international connection

The scientific communities and institutions of the Arab world have evolved a complex set of relationships with foreign countries. To begin with, most scientific manpower engaged in research have relationships with scientists in Europe, the USSR or US. A number of scientific unions already exist in the Arab states and many of these are members of the International Council of Scientific Unions (ICSU). Egyptian scientific councils belong to most of the ICSU councils, and research councils in roughly half the Arab states are members in one or more of the ICSU councils.

Numerous research institutions in the Arab world have been established through bilateral agreements. The nuclear research centres in Baghdad and Cairo, and the bilharziasis research institute in Cairo were established by the USSR and Federal Republic of Germany respectively. In 1942, the US Navy established in Cairo the US Naval Medical Research Unit Number Three (NAMRU-3) which by 1977 had grown to 26 military personnel and 206 civilians (203 from Egypt). NAMRU-3 has played an active role in medical research in Egypt. In 1976, the US and Saudi Arabia signed an agreement through which the US is to assist Saudi Arabia to establish the Saudi Arabian National Centre for Science and Technology (SANCST). The US and Saudi Arabia also have a joint five year $100 million research programme in solar energy.

UN agencies have been involved in a number of different ways in the application of science and technology in developing countries. UN support to scientific research activity, or to the strengthening of indigenous technological capabilities of Third World countries, consumes no more than 1.5% of the $3 billion UN budget. The Arab world share of these $45 million is of the order of $1-2 million.

The number of scientific and technical meetings in the Arab world has been steadily increasing. The Cultural Department of the League of Arab States sponsored these meetings in the fifties. Since then the Arab League Educational Cultural and Scientific Organisation (ALECSO) has been instrumental in convening them. The growing number of scientific associations and societies have also been actively organising scientific meetings.

Likely trends

On the national and regional level, a large number of small scale but steady changes have been registered. New institutions and new universities are created annually. The position that Egypt (with 25% of the population of the Arab world) held in 1950 with virtually the only Arab centre for scientific work (more than 80% of Arab publications in 1950 were by Egyptians) has been changing steadily. In 1967 Egypt produced 63%, and in 1976 55%, of Arab publications; by the year 2000 Egypt's share will be below 40%. This reduction is not due to a decrease of activity in Egypt but rather to an expansion in the other Arab states. For example, the combined output of Kuwait, Libya and Iraq (11% of Arab world population) which was 8% in 1967 increased to 14% in 1976. It is likely that the share of these three countries would have increased to 30% in the year 2000.

The internal movement of scientific manpower between Arab states has been steadily increasing. This has made the development of social and educational services in Arab oil producing countries at a high rate possible. During the past decade most of the increases in the allocations for R&D in the Arab world have taken place in oil producing countries; elsewhere, little increase in funding is recorded.

The Arab scientific communities, the educational systems, their immediate environments, their relationships to the users of technology, and the resources of the Arab states have all been undergoing far-reaching changes. It is very difficult at this moment to identify clearly the new directions that are emerging. On the surface, it would seem that the traditions of the past three decades are firmly entrenched. This can be seen from the CASTARAB 1976 meeting in Rabat, the 1978 Kuwait Fund for Arab Economic Development feasibility study on the proposed Arab Fund for Scientific and Technological Development, as well as the national papers submitted to UNCSTD. Nevertheless a large number of indicators point to the possibility of major departures. Since the early fifties, the production of scientific and technical manpower has been expanding at an exponential rate, with a doubling time of about 5.3 years. If this rate continues, the one million university graduates of 1978 would have grown to more than 12 million by the year 2000. The 24,000 foreign trained doctorate holders could reach a level of 150,000–250,000 by the year 2000.

Meanwhile, the capacity of the US and Europe to drain unwanted high level manpower has been reduced by the economic recession registered there. Furthermore, the size of Arab technical manpower is rapidly reaching a level that makes likely drains an insignificant portion of the total pool of educated manpower. The absorption of this enormous manpower cannot be achieved without effecting major structural changes in current practices of planning and executing development projects. All Arab countries are undergoing rapid social and economic change. Ministries, institutions, industrial installations, and transport systems are all being developed at a high rate. But many developmental projects are being executed in a manner that does not utilise existing technical skills or institutions. This leads to a low level of employment generation within the Arab countries themselves. An interesting comparison provides an indication of the extent of this fact: Imperial Chemical Industries (ICI) provides direct and indirect employment in the UK comparable to that of the hydrocarbon sector in the Arab world; but the sales of Arab oil are roughly ten times those of ICI. Obviously the only way in which the five million graduates in the applied and basic sciences are going to find employment is if the present technological dependence is reduced through the use of indigenous firms and institutions. Technology policies will certainly assume increasing social and political importance during the next two decades. The very stability of Arab countries will depend on the development of employment-generating technology policies.

A profound religious and ethical rejection of the stark contrast between wealth and poverty pervades the Arab world. This has so far led to the establishment of a number of Arab funds (national and regional) that aim to assist sister countries in their development. Finally, the difficulties and mechanics of development are becoming clearer, the social and political cost of inaction is growing increasingly evident, the enormous resources of the Arab world are gradually coming under the control of local institutions and governments. What, when and how a zipper action will operate to cohere all of these disjointed resources is difficult to forecast. But when it does happen the scientific communities of the Arab world will be called on to play a leading role. □

Mountains out of molehills

KENNETH MELLANBY

One of the less attractive habits of our species, *Homo sapiens* (sic), is the way in which we attribute our unpleasant behaviour patterns to other animals. Thus the human glutton is described as a pig or a hog, and the term swine is used to cover a multitude of our sins. Male chauvinists are pigs, and their women bitches when they assert their rights, cows when they do not, and cats when they criticise other women. Occasionally diminutives like kitten are used more affectionately, but as a rule the comparison with an animal is a derogatory one.

1979 has been called 'The Year of the Mole' — with the mole cast as the villain of the piece. Thus while the majority of viewers could not make head or tail of the BBC television serial *Tinker, Tailor, Soldier, Spy*, they did understand that there was a traitor high up in the security organisation who was passing vital information to the enemy — in other words, a 'mole'. This programme had hardly left our screens when we had the real life case of Anthony Blunt, a distinguished art historian and advisor on pictures to the Queen, who confessed to having been a Communist agent passing information to the Russians when he was attached to the British secret service. He was the mole *par excellence*. Then for some years British Leyland, the ailing motor manufacturer, has had its troubles exacerbated by the actions of a Communist shop steward, commonly called the 'red mole'.

Yet the furry little insectivore *Talpa europaea*, has little in common with a spy or a disruptive agitator. It must be admitted that it is equally unlike the loveable and friendly animal featured in Kenneth Grahame's delightful children's book *The Wind in the Willows*, and presented so vividly on stage in London each Christmas by the redoubtable octogenarian actor Mr Richard Goolden in the play *Toad of Toad Hall*. Moles do indeed live underground in burrows, but their most striking characteristic is their solitariness. Rudyard Kipling wrote, in this case with reasonable accuracy, about 'The Cat which Walked Alone', but the mole is very much more independent than the most self-centred domestic pussy.

In fact it is difficult to see anything in the life history of the mole in common with that of a spy. For almost the whole of their lives, both males and females occupy their own separate sections of underground tunnell, and spend the time in sleep or in running up and down picking up worms and insects which have dropped into the burrow. Should a mole be foolish enough to invade another's territory, it is usually driven off at once. Except for a brief period once a year when the female accepts the attentions of the male, when mole meets mole there is at once open hostility and even bloody combat. There is nothing subtle, deceitful or underhand in the behaviour of *Talpa*.

Gardeners who suffer from the appearance of molehills on their carefully manicured lawns may perhaps be excused for saying nasty things about the culprit, but at least they must admit that by making its presence so noticeable the burrower is hardly being secretive or underhand. So should we not try to find some other zoological sobriquet for a traitor or an industrial troublemaker? I fear that any suggestions will arise the wrath of some other mammalologist involved with the libelled species.

Finally, I find that I am, unwittingly, involved in an attempt to cash in on the current interest in moles. I read in the London newspaper *The Financial Times* that: "Collins the publishers tell me that they have had some discussions about expanding the title of Professor Kenneth Mellanby's book *The Mole* to *The Mole — the Blunt Truth*".

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CORRESPONDENCE

Dismissal of Professor Scheer was justified

SIR, — As a member of the federal state government of Bremen I would like to make the following remarks to correct the letter from Uwe Lahl and W. Thiemann of Bremen University (27 September, page 252).

The Senate of the Freie Hansestadt Bremen, the federal state government, has not pursued the dismissal of Professor Jens Scheer from the Bremen civil service because of his political and scientific activities involved with his criticism of the nuclear politics of the Federal Republic of Germany. The Freie Hansestadt Bremen, even the Senate itself, is in fact taking a decidedly critical position regarding the nuclear politics of the Federal Republic of Germany.

Nor has the Bremen government pursued the dismissal of Professor Scheer primarily because he belongs to a non-prohibited political party (the KPD; the Communist Party of Germany). The real reason which has tipped the scales towards disciplinary action against Professor Scheer is rather as follows.

Professor Scheer advocates in public and in Bremen University, while practicing his public duties and abusing his official position, the removal of our free democratic constitutional state of the Federal Republic by violence, and the erection of the dictatorship of the proletariat in its place; this in reality would be the dictatorship of a small elite. In making this aim (of violence) the declared object of his public service activities he abuses the official position given to him.

By the way Professor Scheer has advocated the use of violence not merely verbally, he has already been sentenced for taking part in violence against persons dissenting from his political opinion.

Therefore one cannot reasonably talk about the Bremen State trying to bring scientists under political control by this disciplinary action. Professor Scheer can make use of his constitutional rights in every allowable way. But he cannot legally claim the violent destruction of this state, and at the same time accept payment by the state. All civil rights remain guaranteed to Professor Scheer as a citizen and a scientist of this republic.

The Bremen state government is anxious to learn from German history. The first German Republic of Weimar allowed civil servants to challenge the state which they were sworn to serve, and in the end even to bring about its ruin. The Federal Republic of Germany and especially the German Social Democrats to which I belong have learned their lesson from Weimar.

Finally, the statement "He was not allowed a complete defence" is spun out of thin air. He and his lawyer had every opportunity to plead their position, and they did use it.

Yours faithfully,

FRANKE

Freie Hansestadt
Bremen, FRG

The Sirius Mystery

SIR, — Readers of *Nature* may well be aware from the review of my book *The Sirius Mystery* in the issue of 17 June, 1976 (pages 617–8), that "the problem of the remarkable knowledge of the Dogon people of Mali", referred to by Michael Rowan-Robinson in his review of Carl Sagan's *Broca's Brain* (8 November, 1979, pages 176–7), was first brought to public attention by myself. Although in his review Rowan-Robinson does not mention me by name, and in his book Sagan barely refers to my seminal position with regard to public knowledge of this material except in passing, anyone truly interested in this subject is perfectly aware that they are referring to myself when discussing this matter.

It would be inappropriate for me to discuss Sagan's own errors here. But I would like to reply to statements made by Rowan-Robinson, who says that "Sagan has performed a useful service in collecting together examples of public gullibility and demonstrating how a mixture of deliberate fraud and uncritical thinking has allowed millions to be bamboozled . . .". He says this immediately after discussing my material.

I strongly object to the inference that I have been guilty of fraud in bamboozling a gullible public, and as for "uncritical thinking", the many serious and thoughtful reviews of my book in this very journal as well as others of distinction testify to the fact that although the implications of my work have aroused controversy, it is not widely thought, nor do I obviously think myself, that "uncritical thinking" is a fault of which I have been obviously guilty, especially as my conclusions were all explicitly hypothetical.

The anthropologists who have spent the 48 years from 1931 living with the Dogon tribe are agreed that the knowledge of the Sirius system which they possessed could not have come from Europeans, who in any case had themselves only discovered the superdense nature of Sirius B in about 1926. Knowledge of Sirius B amongst the Dogon goes back hundreds of years, as shown by physical evidence, some of which was even held up in front of television cameras by Dr Germaine Dieterlen, who insisted that it was 400 years old, and said that the European contact theory (espoused by Sagan) was "absurd".

But I do not wish to open this controversy again here. It is in the context of saying that Sagan has shown that the Dogon information "could all have been learnt from contacts with Europeans" (which is untrue) that Rowan-Robinson does me less than justice by saying this is better than it having "been learnt from little green men in UFOs". I have never at any time suggested this; I went out of my way in my book to say that I do not believe that UFOs are extraterrestrial craft of any kind, or connected with my subject, and Rowan-Robinson's remarks are therefore a slur against my intelligence and quality of work which I most strongly resent.

Rowan-Robinson also betrays an indefensible bias against the serious possibility that our planet has at some time in its history been visited by intelligent extraterrestrials. What is so remarkable about this *per se*? Are we so incapable of speculating about these matters that we deny them solely because such a visitation seems to us extraordinary? The electric light bulb would have seemed extraordinary to Plato and Aristotle, but does that rule out the possibility of its eventual invention? This is the kind of inverted logic demonstrated by those whose minds are too small for large notions.

Yours faithfully

ROBERT K.G. TEMPLE

Sutton Mallet, Bridgwater, Somerset, UK

Standardisation of reference citation

SIR, — A significant part of a scientist's time is spent in drafting manuscripts for publication. Part of this task involves the careful listing and checking of references. At present, most journals have their own distinctive style and even basic references have to be rearranged with considerable waste of time and effort as every new paper is drafted. Some idea of the variability of reference citation can be seen if the bibliographies of the following internationally recognised marine science journals (first published in sequence between 1953 and 1979) are examined: *Deep-Sea Res., Mar. Biol., J. exp. mar. Biol. Ecol., Estuar. Cst Mar. Sci., Oceanol. Acta., J. Plankton Res.* One conclusion that can be drawn from such an examination is that the variability of reference citation is increasing with time.

Some progress has been made towards international standardisation with the introduction of the following international standards: ISO 4-1972 (E), Documentation — International Code for the Abbreviation of Titles of Periodicals and ISO 833-1974, Documentation — International List of Periodical Title Word Abbreviations. (See also BS 4148 Part 1: 1970 and BS 4148 Part 2: 1975.) The establishment of this international standard for over five years does not seem to be acknowledged by many editors (including *Nature's*) who still recommend the fourth edition of the *World List of Scientific Publications* for journal abbreviations, a list which was last published in 1963 to 1965! In the absence of a properly updated world list and prior to the publication of the new international standard,some journals (such as *Journal of the Marine Biological Association of the United Kingdom*) have reverted to printing the full titles of journals.

There is so far no international agreement on bibliographic references, rather the opposite, as the American National Standard ANSI 239.29-1977 and British Standards BS 1629:1976 and BS 5604:1978 differ considerably from each other in their recommendations and from each of the journals cited above. Major differences between the two national standards are the use of capitals, italics and bold face in the British Standard in contrast to a single type face and more complicated punctuation rules in the American standard. The 'Harvard' system with the year of publication following the author and a form of the 'Numeric' system as used in *Nature* are the two formats recommended in the new British Standards BS 5605:1978 which was specifically prepared for editors and authors of articles in journals and books. The 'Harvard' format comes closest to that now used by most journals. The simpler punctuation of this standard is also preferable, although the adoption of a simple typeface as used in the American system may be more compatible with computerisation of references.

A standard for abbreviated references, as used for instance in *Nature*, is only given in ANSI 239.29-1977 restricting information to the journal title, volume number, pages and year of publication. This is not sufficient for scientific journals as one needs to assess immediately the 'pedigree' of a particular piece of work by reference to the original authors and if possible an abbreviated title.

Publication of an international standard for bibliographic references in scientific journals is overdue. Major publishing houses, editors of journals and scientists should be consulted in this standardisation process as well as librarians and national standardisation authorities.

Yours faithfully,

P.C. REID

Institute for Marine Environmental Research,
Plymouth, UK.

NEWS AND VIEWS

Pain, enkephalin and acupuncture

from Shin-Ho Chung and Anthony Dickenson

DURING the past few years, there have been enormous strides in understanding the neural mechanisms underlying the perception of pain. From what we now know, it is easy to see why the mechanism of pain perception has remained enigmatic for so long. Unlike the other senses, there seem to be no cortical centres which are solely devoted to processing pain, nor is there a discrete fibre bundle, which can be severed to alleviate chronic pain. Indeed, the system seems to be elaborately designed to ensure that it is fail-safe, with an added feedback loop which, when activated, reduces the intensity of persistent pain.

It is now firmly established that small-diameter fibres, whose endings are distributed throughout the body, including skin, muscles and viscera, respond specifically to painful stimuli. These fibres, on entering the spinal cord, branch profusely and form synapses in the superficial layers of the dorsal horn, mainly in the marginal zone and the substantia gelatinosa[1]. Here the fine terminals of axons and dendrites, together with collaterals of ascending and descending fibre tracts, form a dense jungle of neuropil, the precise wiring diagram of which has yet to be elucidated. There are two distinct groups of spinal neurones which respond to painful stimuli, one located in the superficial layer (lamina I) and involved mainly in transmitting sharp pain, and the other in the deeper layers of the dorsal horn (laminae IV and V) responding to intense pain[2]. Through a dense meshwork of short and long axons, these cells transmit messages further centrally. Some of these axons terminate within the spinal cord[3], whereas others ascend to the brainstem reticular nuclei[4]. The longest bundle proceeds to the thalamus[5,6], accompanied by other axons arising from the brainstem. In stark contrast to the other senses, the neural organisation of the pain system is characterised by elaborate feedback networks. Terminals of at least three descending fibre tracts, which originate from the brainstem nuclei, form synapses in the dorsal horn[7]. In addition numerous short axons of spinal neurones traverse a few segments, their terminals interlacing with the dendrites of pain cells located further rostrally[1].

The interplay between the incoming pain messages and the descending influence from the brainstem has recently been the focus of intensive investigation. In 1964 Taub[8] first discovered that spinal neurones responding to painful stimuli could be silenced when certain areas of the brainstem were stimulated electrically. This initial finding of descending inhibitory pathways, confirmed subsequently by others, has recently been extended. The physiological effect on spinal neurones of electrical stimulation of the brainstem is now known to be confined to cells responding to pain, especially those in laminae IV and V[9-11]; other neurones nearby which convey, for example, information concerning touch or temperature, are uninfluenced by this manoeuvre. Careful mapping has revealed that there are discrete nuclei in the brainstem, stimulation of which produces potent neuronal inhibition and behavioural analgesic effects; among these are the nucleus raphe magnus and the periaqueductal grey[10-13]. The raphe magnus is known to be the origin of certain descending fibre tracts[14,15], but the periaqueductal grey, which is located along the length of the aqueduct, seems to exert its influence indirectly on the dorsal horn through the nucleus raphe magnus[16,17].

The analgesic effect of morphine and related compounds can now be understood in terms of their inhibitory influence on the transmission of pain. For reasons which are not yet entirely clear, morphine reduces the efficiency of synaptic transmission between pain fibres entering the spinal cord and their target cells in the dorsal horn[18-20]. A small quantity of morphine injected into the substantia gelatinosa reduces sensitivity to pain, when tested either behaviourally[21] or electrophysiologically[22]. The action of morphine, however, is also mediated by the descending inhibitory system of the brainstem. From numerous studies involving gross lesions, microinjection of morphine, and biochemical mapping of the distribution of opiate receptors, the same conclusion emerges; the cells in the periaqueductal grey and the nucleus raphe magnus, richly endowed with opiate receptors, detect the presence of morphine and transmit this message down to the dorsal horn of the spinal cord[23,24]. Here the terminals of the descending fibres release serotonin[25], which, through mechanisms yet to be discovered, interferes with the excitatory synaptic processes between the primary pain fibres and spinal neurones[26]. This inhibitory modulation on the transmission of pain, the essence of Melzack and Wall's 'Gate Theory'[27], relies entirely on the integrity of the brainstem descending system. If, for example, the raphe magnus or the periaqueductal grey are destroyed, or naloxone (a specific opiate antagonist) is injected into these brain areas, then the analgesic effect of morphine is drastically reduced[23,28-30]. Moreover, both the neuronal inhibition and the behavioural analgesia induced by electrical stimulation of the raphe magnus are reduced by naloxone[31,32]. Why, of all other substances, opiates and opiates alone turn out to be such potent pain-killers is one of the deep mysteries of the brain.

While the neural basis for analgesia was beginning to be understood, two related pentapeptides, known as Met-enkephalin and Leu-enkephalin, were isolated from the brain. These opioid substances, along with other related peptides containing the enkephalin sequence, are present in axonal endings of both central and peripheral nerves. They bear a stereochemical resemblance to opiates and are known to be synthesised locally. Biochemical and immunohistochemical analyses have revealed that those parts of the nervous system directly involved in mediating or modulating pain are especially rich in both opiate receptors and the endogenous opioid substances. Since these discoveries, some of the electrophysiological and behavioural findings obtained with morphine and electrical stimulation of the brainstem have been replicated with

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Shin-Ho Chung and Anthony Dickenson are at the National Institute for Medical Research, London.

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enkephalins[33,34]. All the findings to date show that the action of morphine can be mimicked by enkephalins. Some problems remain however. We want to know, for example, where on the neuronal membrane opiate receptors are located and how the peptides influence the receptors to generate synaptic currents.

If the brainstem inhibitory nuclei are activated by naturally occurring opiates as a result of injury, would it not be possible to mimic this by stimulating peripheral nerve fibres carrying pain messages and so reduce the intensity of persistent pain? The technique of acupuncture, which the Chinese have traditionally used for combating pain, seems to be based on this principle. In a series of elegant studies, Le Bars and his colleagues[35,36] have demonstrated that cells in lamina V of the spinal cord responding steadily to painful stimuli applied to the hind limb can be silenced by activating, either with natural or electrical stimulation, pain fibres from other parts of the body. These lamina V cells are the ones that respond to intense pain, and the reduction of their activity is brought about by the descending inhibitory system from the brainstem and also can be mediated by opiates[37]. On the other hand, the activity of some cells can be inhibited by the local circuitry within the spinal cord[27,38], without involving the brainstem.

Thus, it is now becoming clear that the analgesic effect of acupuncture is the result of interaction between impulses from the origin of pain and afferent impulses from the acupuncture point[39], and that this interaction takes place at different levels of the central nervous system[40]. For acupuncture to be effective in relieving pain, however, the involvement of the nucleus raphe magnus and endogenous opiates is required. There are several independent lines of evidence for this assertion. Pomeranz and his colleagues[41,42] have shown that the analgesic effect produced by acupuncture in animals is abolished if naloxone is injected. Several clinical studies also note that strong acupuncture is most effective for alleviating chronic pain in humans[43–46], and the analgesia so produced is antagonised by injection of naloxone[43,45,47]. In retrospect, it is clear that the Chinese have been at the forefront of research into pain. They have known for several years, that the nucleus raphe magnus[48], the serotonergic system[49], and inhibitions of lamina V cells[50] are intimately involved in producing the analgesic effect of acupuncture. To the Western mind, it has been inconceivable until now, exactly how placing a single needle into a certain point of the body could produce such a potent amelioration of pain elsewhere in the body.

There is a glimmer of hope that, in the near future, new methods of controlling pain and new analgesics without addictive properties may be found. But the fundamental question of how we perceive pain still remains to be answered. Pain, after all, is a conscious awareness, and so involves the full participation of higher cortical centres. On the role of the cortex in perceiving pain, we remain totally ignorant. It is towards the elucidation of the interaction between perception and transmission of pain that future research must be directed. □

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Footprints of the Sun

from Bevan M. French

WHAT has the Sun been up to lately? The question is becoming important both scientifically and socially, as more evidence appears that the Sun is (and has been) a variable star. Astronomers worry about the Sun's variability as a clue to understanding stellar behaviour. Everyone else is (or should be) concerned about what future solar variations might do to our weather, climate, food production, and civilisation. Understanding the Sun's past may help us predict its future behaviour a little better.

Where are the records of the Sun's past? Almost everywhere in the Solar System, if the results presented at the Conference on the Ancient Sun last October*, are any indication. Traces of solar behaviour have been left on many worlds, and the list of materials that hold assorted obscure parts of the record reads like a planetary jumble sale: historical documents, tree rings, pollen collections, deep-sea sediments, glacial deposits, spacecraft measurements, Marine and Viking photographs of Mars, polar ice cores, Apollo lunar samples, and meteorites. In each material the solar record, while readable, is often incomplete, ambiguous, or model-dependent. A greater problem in putting the pieces together is the lack of communication between the unusually wide range of specialists involved — solar astronomers, historians, nuclear physicists, glaciologists, tree-ring daters, and the geologists, geochemists, and physicists involved in moon rock and meteorite studies.

The goal of the Conference was communication: to introduce each participant to the data available from other fields. It succeeded extremely well. Solar astronomers described for geologists the current state of theories (or the lack thereof) about how the Sun works. Others reviewed the information about solar variability that has been extracted from terrestrial climate records, from historical records, and from lunar samples and meteorites. These reviews set the stage for detailed examinations of solar variability over three timescales: short-term (0–10^2 yr), intermediate (10^2–10^5 yr), and long-term (10^5–10^9 yr).

There is clear evidence of short-term variability in the present-day Sun, even though detailed records of such things as solar flare activity and the solar constant (S) go back less than 50 years and reliable sunspot records cover less than two centuries. Short-term (hours to days) changes are observed in the solar radio, ultraviolet, and X-ray outputs (W.J. Borucki, NASA-AMES Research Center; P.V. Foukal, AER Inc., Cambridge, Massachussetts), and in the flux, velocity, and He/H ratio in the solar wind (A.J. Hundhausen, HAO, Boulder). Variations in the composition of solar flare particles have been measured by spacecraft (R.A. Mewaldt, Caltech, Pasadena), and measurements of lunar samples indicate significant variations in the proton flux on the order of decades (R.C. Reedy, Los Alamos Scientific Laboratory). The modulation of galactic cosmic rays by the 11-yr-cycle of the solar activity has been known for some time, but studies of cosmogenic radioactivities in meteorites that fell during the period 1967–78 (J.C. Evans, Johnson Space Center, Houston) indicates that the modulation is about three times as intense as expected. Despite these variations, the solar constant (S) has apparently remained constant to within about 1%, but recent spacecraft measurements suggest that variations of a few tenths of a percent have actually been detected. Even such small changes have significant effects on the Earth's climate.

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*Held at Boulder, Colorado on 16-19 October 1979. A limited number of abstract volumes are available from: Administrative Office, Lunar and Planetary Institute, Houston, Texas 77058 USA. US requests should include \$2.00 to cover handling and mailing costs. Non-US requests should include \$1.05 for return by surface mail, \$4.25 for airmail return. The Proceedings of the Conference will be published in late 1980 by Pergamon Press.

In the intermediate period (10^2–10^5 yr), historical records become spotty, and scientists are dependent on the solar record in natural materials (both terrestrial and extraterrestrial). The Maunder Minimum, a period of reduced solar activity (1645–1715 AD) characterised by a virtual absence of sunspots, has now been well established by a combination of historical records (J.A. Eddy, HAO, Boulder) and ^{14}C studies of bristlecone pine tree rings (M. Stuiver, University of Washington, Seattle); the effects of reduced solar activity at this time may also have been detected in iron meteorites (O.A. Schaeffer, SUNY, Stony Brook). Earlier periods of both reduced and increased solar activity are also seen in the bristlecone pine records back to about 5,000 b.p. (Stuiver).

A debate about possible variations in solar flare intensity during the past 10^4 years has been generated by the record of solar flare particle tracks preserved in rocks and small crystals collected from the surface of the Moon. Comparative counts of solar flare tracks and the microcraters formed by cosmic dust particles suggest that both the dust flux and flare activity have been constant for about 5×10^5 yr (E. Zinner, Washington University, St Louis), but the possibility of higher flare activity about 10^4 years ago is still being considered (H. Zook, NASA Johnson Space Center, Houston). One suggestion of higher flare activity is the excess amount of ^{14}C detected in lunar surface soils (E.L. Fireman, Smithsonian Astrophysical Observatory). This could have been produced by solar flares. The alternative explanation, that the ^{14}C was produced in the Sun and implanted by the solar wind (Fireman), raises theoretical problems about just how ^{14}C is now being made inside the Sun.

Long-term variability (10^5–10^9 yr) involves the problem of what the 'early Sun' was like soon after its formation. Until about a decade ago, this problem area was inhabited entirely by theorists. Now a new group of scientists is extracting information (and even solar particles) from samples that actually saw the face of the ancient Sun — rock breccias from the lunar highlands and tiny crystals that were compacted into meteorites more than 4×10^9 years ago.

To quote James Arnold's (University of California, San Diego) summary of the Ancient Sun: "Things haven't changed an awful lot." G. Crozaz' (Washington University, St Louis) review made the following points: (1) solar flares were active at least 4.2×10^9 years ago and left particle tracks in meteorite grains exposed to the Sun at that time; (2) the solar flare energy spectrum (measured in lunar samples, meteorites, and a glass lens from the Surveyor 3 spacecraft) seems to have been about the same for the last 4×10^9 yr; (3) enrichment of heavy elements at low energies (<10 MeV) is seen in both ancient and modern flares; (4) no convincing evidence for higher solar activity during the early history of the Moon (about 3.9–4.4×10^9 yr ago) has been found in lunar samples; (5) there are no apparent changes in the character of galactic cosmic rays during at least the past 50×10^6 yr.

Along with this reassuring evidence of constancy, there are some surprising variations, for which the main evidence is the analysis of ancient solar wind particles trapped in lunar samples over the past 2–3×10^9 yr. The most puzzling effect is a major variation (about 30%) in the ^{15}N/^{14}N ratio of nitrogen in the solar wind, from about −200 permil 2–4×10^9 yr ago to about +100 permil now (R.N. Clayton, University of Chicago; J.F. Kerridge, University of California, Los Angeles), combined with an excess amount of total nitrogen in the ancient lunar soils. Because the Moon contains virtually no indigenous nitrogen, these variations must arise in the solar photosphere, but there is no agreement, and considerable bewilderment, about how such gross isotopic changes can be produced and why they appear in only a single chemical element.

The Conference made possible an exciting introduction, review, and dialogue about the sun's history. What comes next?

- Reworking of current solar theories to include the hard data now available about the ancient and modern Sun. For example, how are such 'proxy' indicators as sunspots and the intensity, energy, and composition of solar wind and solar flare particles related to overall solar luminosity?
- More and better solar measurements, especially of the solar constant (S) and the solar radius. New space missions such as Solar Polar to observe the Sun, and the Venus Orbiter with Imaging Radar (VOIR) to scan ancient planetary surfaces.
- New analytical methods — accelerator measurement of new cosmogenic nuclides such as ^{10}Be, and ion microprobe studies of ancient solar wind implanted in Moon rocks and meteorites.
- Studies of newly available materials that contain important parts of the solar record: micrometeorite spherules from deep-sea sediments; deep cores of polar ice that go back more than 8,000 years; new collections of meteorites that fell in the Antarctic and were preserved in the icecap, some for more than 10^6 yr; old historic falls of iron meteorites that may actually record the Maunder Minimum; and a wide variety of historical records, especially from Oriental countries, that have never been examined in detail.

If the problems of deciphering the Sun's history are still immense, so are the new materials and techniques now available to attack them. Scientists from many different fields have many challenging and exciting things to do before they meet again to talk about what the Sun has been up to.

Bevan M. French is NASA's Discipline Scientist for Planetary Materials. He is also author of The Moon Book *(Penguin Books, 1977), a nontechnical review of moon rocks and lunar science.*

Drug metabolising enzymes

from A.E.M. McLean

THE mamalian liver responds to an influx of chemicals by making more of the enzymes that oxidise and conjugate the majority of foreign chemicals. Induction of enzyme synthesis by carcinogenic hydrocarbons was shown in the rat by Alan Conney (Hoffman La Roche, Nutley) more than 20 years ago and he returned to a Ciba symposium in London recently* to describe induction of drug metabolising enzymes in man by dietary factors. High protein diets, charcoal broiled steaks, and brussel sprouts all have potent inducing effects.

Discussion ranged on the relative importance of natural compared with synthetic inducers such as drugs and polychlorinated biphenyls in the environment. The importance of genetic factors compared with environmental inducers in controlling drug metabolism, was brought up by R. Smith (St. Marys Hospital Medical School, London) who presented information about the different products of metabolism of debrisoquine and phenacetin. He pointed out that the same plasma half life for a drug might be, and often was achieved by quite different genetically determined pathways of metabolism, so that it was important to know not only the rate of disappearance of a drug, but what products were formed.

The need to know the fate of toxic materials was a recurrent theme. Only by measuring the exact fate of carcinogenic compounds like benzo (a) pyrene was it possible to see what influence exposure to inducing compounds, which can increase both toxic and detoxicating pathways, would have on toxicity. Most carcinogens require metabolism to their active form before they will attach DNA, and the sweet potato poison, ipomeanol, similarly needs oxidative activation in the Clara cells of the lung before it causes acute lung damage (M. Boyd, National Cancer Institute, Bethesda). The pathways of activation and detoxification are so numerous that it is difficult to predict whether previous exposure to inducers will increase or

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*The Proceedings will be published as Ciba Foundation Symposium 76 under the title of 'Environmental Chemicals, Enzyme Function and Human Disease' by Excerpta Medica, Amsterdam in Summer, 1980.

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decrease the toxic effects of other chemicals.

A. Breckenridge (University of Liverpool) described the effect of inducer drugs in rendering low dose oral contraceptives inactive. It is not clear whether brussel sprouts would have the same effect. A. McLean (University College Hospital Medical School) found no detectable effect on mortality of people with epilepsy of the inducing effects of anticonvulsant drugs. He suggested that the next line of investigation in toxicology should be to examine the response of cells to attack by activated toxic molecules. E. Farber (Toronto) also suggested that too much attention had been paid to the chemistry whereby a molecule of toxic material was activated and interacted with cell macromolecules. Not enough thought had been given to the response of the cell. If 10% of the genome was devoted to response to adverse conditions it would be a reasonable proportion, and leave an enormous range of built-in responses to toxic attack. Carcinogenesis might be the end stage exaggeration of such an adaptive response to a chemically unfavourable environment. A system for development of tumour nodules in a few weeks promised to be a favourable model for analysis of the cell response to carcinogens.

T. Connors (MRC, Carshalton) and J. Higginson (IARC, Lyon) started and ended the meeting by reference to the need to bring human and laboratory experience together, the need to understand tumour promotion as well as carcinogen activation, and the need to combine biochemistry and epidemiology. □

A.E.M. McLean is Reader in Toxicology at the University College Hospital Medical School, London.

The ecology of the footprint

from Peter D. Moore

THE ecological effects of recreational pressures on mountains, sand dunes and wetlands are reasonably well documented, and a great deal of work has been carried out and much has been written on the subject of trampling. What is surprising, in view of this, is that so little is known of the ecology of the footprint.

That the footprint has a distinctive ecology of its own was clearly demonstrated by some accidental experiments of Harper, Williams and Sagar some 15 years ago (*J. Ecol.* **53,** 273; 1965). They were in the process of examining the germination characteristics of three plantain species (*Plantago lanceolata, P. major and P. media*) in open plots, when an oversight led to the trampling of their plots by pigs involved in other experiments. Groups of *Plantago* seedlings appeared wherever a hoofprint had been made. Evidently the alteration of microtopographic, and hence microclimatic and microhydrological features of the soil surface by the impact of the hoof, had strongly influenced the germination and establishment of the plantains. Harper *et al.* developed these observations into a series of experiments in which they confirmed the importance of microtopography in seed and seedling behaviour.

Since then, many attempts have been made to examine the microclimatological changes associated with trampling, both above and below the soil surface. A range of soils, from chalk (Chappel *et al. J. appl. Ecol.* **8,** 869; 1971) to sand (Liddle & Grieg-Smith *J. appl. Ecol.* **12,** 893; 1975) have been looked at. But in these studies the emphasis had changed from the single footprint to the overall influence of continuous trampling in creating tracks and paths.

One habitat in which the individual footprint survives and remains a scar on the surface for a considerable time is the *Sphagnum* bog. All mire systems are sensitive to trampling for, instead of a mineral soil being compressed, the plant body itself and the organic detritus underlying it, receives the direct impact of the foot. Relatively deep depressions are formed which may fill with water, modifying the microenvironment and creating small scale pool successions. Slater and Agnew (*Biol. Conserv.* **11,** 21; 1977) have investigated the development of footprints at Borth Bog in west Wales. They estimated that the 1,890 visitors to the bog each year (mainly researchers and students) displaced a total of 4,228 m^3 of the bog surface by trampling. Taking a single footprint, about 50% of the displaced volume was restored in the first month, largely by the elastic response of the bog moss carpet. Subsequent recovery was far slower, being about 90% complete only after 18 months; often it took 30 months for all traces of the footprint to disappear. One species which seemed particularly well adapted to colonisation of disturbed sites was the white-beaked sedge (*Rhyncospora alba*), which, the authors contend, may increase the susceptibility of the vegetation to fire, adding to the ecological complications caused by trampling.

A further example of the distinctive ecology of the footprint has now been reported from the edges of a glacier in northern Norway by Theakstone and Knighton (*Arctic Alpine Res.* **11,** 353; 1979). The level of a lake at the outlet of the Austerdalsisen glacier has been lowered 70 m by the construction of a flood control tunnel, and the sediments exposed are now being invaded by terrestrial vegetation. The footprints of reindeer and man in the exposed silts have become colonised by the moss *Aongstroemia longipes.* The depressions are 1-2 cm deep and were first created in the summer of 1976. The colonisations observed in 1977 have persisted into 1978, the moss patches still displaying the shapes of the original foot or hoof prints. One suggestion is that the ponding of water in these hollows may have provided the appropriate hydrological conditions for the moss to establish itself. It is interesting to note that the first British report of *Aongstroemia longipes* was in the Ben Lawers area following soil disturbance as a result of dam construction (Crundwell, *Trans. Brit. Bryol. Soc.* **4,** 767; 1955).

Evidently the ecology of footprints warrants further study. Such research may confirm the old maxim that one plant's depression may be another's opportunity.

Peter D. Moore is a Senior Lecturer in the Department of Plant Sciences, King's College, London.

Transformation and tumorigenesis

from R.E. Langman

AT a recent workshop on transformation mechanisms* M. Cohn (Salk Institute) opened with a plea that the meeting address the question of how many genetically determined events are required to convert a cell from the normal to transformed (tumorigenic) phenotype in the absence of any host surveillance mechanisms. In practical terms the change from anchorage-dependence to anchorage-independence (that is, the cells form colonies in agarose) is almost universally accepted as the assay for transformation. But we know virtually nothing about why colony formation in agarose indeed correlates so well with tumorigenicity, except to note that lymphohaemopoietic cells also normally grow as colonies in agarose, and are not obviously transformed or tumorigenic *per se*. In seeking possible generalisations, Cohn argued that there were few ways a cell could become transformed, one linked to anchorage-independence for non-haemopoietic cells, the other linked to whatever causes lymphohaemopoietic transformation.

I. Weissman (Stanford University) showed that individual lymphoma cell lines from mice produce a virus that binds specifically to that lymphoma, and monoclonal antibodies which block virus binding also block cell growth. Thus, it was argued in this case that virus, by perhaps acting like an antigen, provides the proliferative stimulus for the cell it infects. In general then one might view the lympho-

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*An Armand Hammer Cancer Workshop was held at the Sall Institute on 12-16 November, 1979.

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haemopoietic cells as anchorage-independent by differentiation, but geared to require constant signalling for continued growth, as seen in this viral lymphoma example.

Using the DNA 'transfection' technique, B. Shilo (Massachusetts Institute of Technology) showed that DNA from several cell lines transformed with carcinogens could cause 3T3 fibroblasts to acquire the fully transformed phenotype of anchorage independence and tumorigenicity. The frequency of 'transfection' in these experiments is consistent with the idea that a single dominant gene is responsible for the transformed phenotype; this had been shown previously in the majority of cell hybridisation experiments. Furthermore, transformation of normal recipient cells with the transferred DNA was associated with a unique restriction fragment of DNA. Thus one, or a cluster of tightly linked genes is capable of dominantly causing the expression of the transformed phenotype. The analysis of this 'transforming' fragment and the characterisation of the protein product (if any) will be awaited with interest.

It is well established that the *src* gene of avian sarcoma viruses such as Rous sarcoma virus (RSV) is necessary for expression of the transformed phenotype. As yet it is not quite proven that the *src* gene product alone is sufficient, but the available evidence strongly points in this direction. The 60,000 molecular weight (60K) protein now thought to be coded by the *src* gene is a protein kinase and is very similar to a host cell protein kinase. T. Hunter (Salk Institute) showed that an *in vitro* rabbit reticulocyte protein synthesising system primed with RSV RNA produced a 60K protein that is indistinguishable from the protein identified in RSV-transformed cells. It was also shown that the 60K Src protein is a tyrosine kinase, and that in normal cell extracts phosphotyrosine is a minor component of the phosphorylated amino acids, but that after RSV transformation, the amount of phosphotyrosine, relative to phosphoserine, was increased 5-10 fold. A. Levinson (University of California, San Francisco) reported that the 60K Src protein is tightly bound to, and therefore probably integrated into, the plasma membrane. After mild proteolysis a 56K protein is found free from the membrane, and retains the kinase activity. Attempts to label the 60K Src protein on the outside of the cell have been unsuccessful up to now, and indicate that it is attached to the inside of the plasma membrane. As the Src protein kinase is very similar to the normal cell kinase but is distinct both structurally and in its kinase activity, the suspicion is that the viral Src product initiates transformation by radically upsetting the regulatory functions of the normal cell 'Src' kinase. How this upset of kinase activity leads to transformation is not clear, except that Src protein is required continuously for the expression of the transformed phenotype, as evidenced by temperature-sensitive mutants of *src*.

Although the Abelson leukaemia virus (ALV) lacks an identifiable *src* gene, O. Witte (Massachusetts Institute of Technology) showed that a 120K protein with tyrosine kinase activity is produced from the ALV genome. This kinase differs from the RSV kinase in that it requires Mn^{2+} instead of Mg^{2+} cations, but is similar inasmuch as there is in murine bone marrow cells (the preferred viral target *in*

R.E. Langman is an Assistant Research Professor at the Salk Institute.

Palmdale bulge: fact or fiction?

from Peter J. Smith

SOME curious things have been happening to southern California recently; so much is certain. The only problem is that the curious phenomena that everyone thought were taking place may not have been taking place at all, whereas some quite different curious behaviour may have been going on. Or, what appeared to be certain facts with uncertain explanations now seem to have been uncertain facts with even more uncertain explanations.

Bits of the story have been coming out at intervals for several months now, although the full extent of the problem only become clear at last month's meeting of the American Geophysical Union in San Francisco. The most startling piece of experimental evidence was presented by A.E. Niell of NASA's Jet Propulsion Laboratory, who reported that the distance between his Pasadena laboratory and NASA's Goldstone Deep Space Station some 193 km to the northeast had increased by 20.3 cm in just 7 months. This remarkable measurement was made by repeatedly recording radio noise from quasars simultaneously at the Pasadena and Goldstone radio telescopes, a procedure that can give distance changes between the telescopes to within ± 5 cm; but the expansion has been confirmed in general terms by laser ranging experiments carried out independently by the US Geological Survey. According to J.C. Savage and W.H. Prescott, laser measurements show that between 1974 and 1978 southern California was undergoing a nearly uniform contraction in the north–south direction but that since 1978 there has been east–west expansion with little or no north–south compression. So the laser data for the post-1978 period are consistent with the 1979 radio interferometry.

Unfortunately, however, the laser data as a whole seem not to be in agreement with the behaviour of the so-called Palmdale bulge, the 84,000 km^2 area of southern California which covers the southwestern section of Niell's interferometry baseline and which has undergone well-publicised ups and downs over the past 20 years. The rise of this zone apparently began in 1959, although this was not discovered until 1976 when it immediately set off speculation about an impending earthquake. By 1974 the maximum uplift had reached 45 cm; but since then there has been a remarkably rapid deflation accompanied by a tilt of the whole region to the north, an increase in the horizontal strain and an increase in radon emission — a group of phenomena which has again sparked off seismic speculations. The strange thing is, however, that the behaviour of the bulge is inconsistent with much of the laser-derived information about the larger area. When southern California was undergoing north–south contraction from 1974 to 1978, a simultaneous east–west or vertical expansion was to be expected. But during this period the bulge was actually contracting, which it continued to do even when the north–south contraction stopped. As Barry Raleigh of the US Geological Survey recently noted, "no physical model has yet been proposed which incorporates these apparently inconsistent results."

And maybe no model is necessary, for what if the observations are wrong or perhaps, let us say, not quite what they appear to be? After pouring more than $1 million into the study of a supposedly deflating bulge, this may seem an odd point to make; but D.D. Jackson and W.B. Lee made it at San Francisco in all seriousness. From an examination of the data going right back to 1959, especially those along the profile San Pedro — Los Angeles — Saugus — Palmdale, Jackson and Lee conclude that most, if not all, of the rise centred on Palmdale never in fact took place at all. They find that the altitudes of larger features such as mountains, as indicated by the raw observations, have changed by the same amounts and at the same times as those of the smaller features such as ridges; and although a tectonic cause for such behaviour cannot be ruled out completely, the simplest explanation is the presence of a systematic error, probably in the calibration of the levelling rods.

Many people are simply not going to believe that, at least without much more evidence, if only because of the embarrassment it would cause. Yet there is clearly something going on in southern California. Monitoring continues.

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

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vivo) a 120K tyrosine kinase with properties very similar to the viral kinase. Although this extends the generality of tyrosine kinase activity as a crucial factor in transformation, it is clear that the small DNA tumour viruses such as SV40 and polyoma lack identifiable kinases. T. Benjamin (Harvard University) noted that mutants in the *hrt* gene region in polyoma virus, which overlaps with the regions encoding the big, middle and small 'T' antigens, showed decreased levels of histone acetylation compared with wild-type polyoma. All these findings on viral transformation together point to several pathways leading to an ultimate transformation site, and to the ability of different viruses to tap into these different pathways to yield the same overall result.

Returning to the opening question of how many genetic events are required for expression of the transformed phenotype, it seems clear that one is a possibility, but that how many different events can give this apparently single gene change is less clear although one must suspect that it is greater than one. Epidemiologists have argued for multistage models of *in situ* tumorigenesis, especially for carcinomas where a minimum of between four and six steps is predicted. If one (dominant) or two (recessive) gene level events can give rise to *in vitro* transformation, then the suspicion must be that the remainder of these steps are either spurious because of assumptions about the data and its relation to tumorigenesis, or are due to evasive steps a transformed cell must take in order to survive various surveillance mechanisms.☐

Polarised electrons are going round in circles

from C. B. Lucas

SPIN-polarised electrons are of interest to physicists and biologists at both the high and low ends of the particle energy spectrum. Several recent advances have provided new sources of polarised electrons, and should provide new insights in fields such as neutral currents, surface physics and molecular optical activity.

One way of producing spin-polarised electrons is to take a storage ring, fill it with electrons or positrons, and leave for an hour or so. This recipe for polarising electrons by macroscopic means seems contradictory to long-standing arguments that they cannot be so polarised, for example by passage through a Stern-Gerlach magnet. In that case, the splitting of the beam after passing through an inhomogeneous magnetic field is less than the uncertainty in their position as given by Heisenberg's principle.

However the polarisation mechanism is easily understood if one remembers that an electron orbiting in the magnetic field of a storage ring and emitting synchrotron radiation may flip its spin on emitting a photon. The probability of a transition depends on the spin direction relative to the guiding magnetic field, the positrons becoming polarised in the direction of the field, the electrons in the opposite direction. Since the probability of a spin-flip emission is typically only 10^{-11} that of a normal emission, the polarisation builds up very slowly.

The polarisation effect was first predicted by Ternov, Loskutov and Korovina as early as 1961. Two years later it was shown that, in a typical storage ring, the transverse polarisation should build up in a period of about an hour to a limiting value of 93.4%.

The first experimental confirmations of theoretical predictions were made on electrons in the storage rings at Novosibirsk and Orsay in 1971. In the former, at an energy of 640 MeV, approximately 50% polarisation was measured after about 100 minutes, compared with a calculated value of 66% when depolarisation effects were included. Since then, measurements on several of the world's storage rings have been made, confirming these results, and 76 ± 5% polarisation has been reported from Stanford. (The latest position was discussed at the Argonne conference on High Energy Physics with Polarised Beams and Polarised Targets (*AIP Conference Proceedings* No 51, ed. G.H. Thomas, 1979)).

Another example of a circular approach to this problem is the latest experiment to produce polarised electrons by photoionisation with circularly polarised light. This process is known as the Fano effect, after its originator. In this case, polarised electrons are produced by transfer of angular momentum from the photons to the electrons. The first Fano-effect studies were made in the near ultraviolet with caesium, and were remarkable in producing electrons, almost completely polarised, with wavelengths close to a minimum in the photoionisation cross section.

Measurements in the vacuum ultraviolet require more sophisticated optical techniques. Measured polarisation at wavelengths longer than 140 nm ranges from a maximum of about 50% in thallium to, typically, a mere 15% in lead. Molecules have been even more disappointing, yielding no significant polarisation of the photoelectrons.

Nevertheless measurements have been continued further into the vacuum ultraviolet, at around 100 nm, by a group using the radiation produced by the synchrotron running at 800 MeV at the University of Bonn (*J. Phys. B* **12**, L679; 1979). Preliminary measurements showed that the radiation above and below the plane of the electrons was respectively left and right circularly polarised to at least 80%. When used to ionise xenon, circularly polarised photons produced electrons which were polarised up to 75%, in general agreement with theoretical predictions. Though still not so impressive as the very first measurements in caesium, these results are comparable with the polarisation which could have been obtained if the electrons in the synchrotron had been injected into a storage ring, though the current would then have been many orders of magnitude greater. Hence polarised electrons in a storage ring could be used to produce polarised photoelectrons through the synchrotron radiation! This means that the particle physicist is rapidly losing his interest in the polarised electrons produced by the atomic physicist, since the former has a very intense source of highly polarised energetic electrons (and positrons) accidentally at his disposal; this provides the opportunity to study directly the neutral weak current phenomenon.

The atomic physicist can unfortunately make little use of polarised electrons or positrons at such high energies, and the laws of electron optics prevent a useful well-defined low energy beam from being obtained from one of much higher energy. Of interest, therefore, is the report by a group at the University of Michigan (*Phys. Rev. Lett.* **43**, 1281; 1979) that 1 eV positrons have been produced with at least 20% polarisation. The authors suggest that the principal use of this source to the physicist will be for the study of polarised low-energy positron diffraction from surfaces. For the biologist, interest in this source is connected with the controversial hypothesis that the origin of biomolecular optical activity might be linked to the polarisation of β-particles from radionuclides.

Interest in spin-polarised electrons is burgeoning as the number of different mechanisms for their production has increased rapidly in the past decade; previously, only elastic electron scattering by heavy atoms looked at all useful. Production of polarised electrons by the use of lasers has not yet proved to be particularly important. Developments in multiphoton ionisation of alkali atoms look very promising, since the atom can be pumped into a fully polarised excited state and then photoionised with a second circularly polarised laser beam. The electrons are then not only expected to be almost fully polarised, but also to have the least energy spread of any source of electrons, so that interesting experiments with low energy polarised monochromatic electrons should become possible. ☐

C.B. Lucas is a SRC research fellow in the Department of Physics, Royal Holloway College, University of London.

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REVIEW ARTICLE

Intermediate filaments as mechanical integrators of cellular space

Elias Lazarides

Division of Biology, California Institute of Technology, Pasadena, California 91125

Five chemically distinct classes of intermediate filaments can be identified within higher eukaryotic cells. Each class is characteristic of a particular cell type. These filaments may function to integrate mechanically the various structures of the cytoplasmic space in a way that is tailored to the differentiated state of the cell.

FOR some years electron microscopy has identified a major filamentous system in the cytoplasm of higher eukaryotic cells that is distinct from actin filaments, microtubules and myosin filaments. As their characteristic diameter of 100 Å is between that of the smaller actin filaments (60 Å) and the larger microtubules (250 Å) and myosin filaments (150 Å), this new filamentous system has come to be known as the 'intermediate filaments'. Intermediate filaments were at first widely regarded as disaggregation products of either microtubules or myosin filaments and as a result they were neglected. But new biochemical and immunofluorescent techniques have recently established intermediate filaments as a distinct fibrous system composed of chemically heterogeneous subunits. The subunit structure defines five major classes of intermediate filaments that can be distinguished both biochemically and immunologically: (1) keratin (tono) filaments, found in epithelial cells and cells of epithelial origin; (2) desmin filaments, found predominantly in smooth, skeletal and cardiac muscle cells; (3) vimentin filaments, found in mesenchymal cells and cells of mesenchymal origin; (4) neurofilaments, found in neurones; and (5) glial filaments, found in all types of glial cells. It is common to find two of these classes co-existing in the same cell. This review highlights the most recent studies which have led to these conclusions and focuses on certain observations that shed light on the cytoplasmic function of these filaments. From these studies, it seems that intermediate filaments have a very important role in the mechanical integration of various cytoplasmic organelles.

Keratin filaments

Keratin filaments are perhaps the most atypical of all intermediate filament classes. Mammalian keratins are a group of intracellular α-fibrous proteins associated in a filamentous form (tonofilaments) with an approximate diameter of 80 Å. They are actively synthesised by the inner, living cell layers of the mammalian epidermis and represent its major differentiation product. Eventually, the keratins form the bulk of the outer, fully differentiated, dead, stratum corneum layer[1].

In addition to being the major differentiation product of epidermal cells, keratins are present in certain other stratified squamous epithelia[2,3], but they are absent from cells of mesenchymal origin (Table 1). Antibodies to the mammalian keratins have been used in immunofluorescent studies to identify many epithelial cells in culture or in tissue sections[4–7]. Generally, these antibodies show a wide cross-reactivity among species, indicating that different keratins are immunologically related. In cultured epithelial cells, the keratin filament bundles which react with the antibodies form an elaborate array throughout the cytoplasmic space (Fig. 1). Electron microscopy shows that bundles of keratin tonofilaments often terminate in membrane-bound desmosomes[4–7].

Keratins are insoluble in neutral aqueous buffers, but can be extracted in the form of a multichain unit[8,9] at low *p*H or by denaturing solvents (for example, 8 M urea). A reducing agent is also required for the efficient solubilisation of keratins from the terminally differentiated cells. Electrophoresis in denaturing gels separates keratin into a family of polypeptides with molecular weights (MW) ranging from 40,000 to 65,000 (refs 10–14 and refs therein). Keratins extracted in the presence of urea from the living cells of epidermis[11–13] or from cultured human keratinocytes[14] are composed of six to seven major polypeptides which have MWs of 47,000 to 58,000. Dialysis of the urea extracts against a dilute salt solution results in the spontaneous formation of filaments without any obvious requirement for a specific cofactor(s) such as divalent cations and nucleoside triphosphates. The reconstituted filaments show the same polypeptide composition, morphology, characteristic diameter of 80 Å and X-ray diffraction patterns as native filaments[10–14]. Extraction and reconstitution experiments suggest that most keratins of living cells exist in a reduced form that favours polymerisation and become increasingly cross-linked by intermolecular disulphide bonds as differentiation proceeds[12–14].

Most combinations of two or three purified keratin polypeptides polymerise *in vitro* into filaments of the same general structure as native keratin filaments. With two polypeptides, reconstitution is in the precise molar ratio of 1 : 2 whereas with three polypeptides equimolar reconstitution is the rule[12]. These ratios are maintained even in filaments polymerised from mixtures containing disproportionate amounts of polypeptides, indicative of a highly specific interaction between the various keratin polypeptides[12]. X-ray diffraction data had also indicated that native epidermal keratin filaments are composed of a fundamental three-chained structural unit[15–17]. As the keratins are composed of six or seven polypeptides, many triple-chained units of similar structure but of different polypeptide composition must be possible. Thus, several different triple-chained units may assemble to form a single filament[12]. If this is the case, then both the reconstituted and native filaments are likely to be heterogeneous with respect to polypeptide composition. Such filament heterogeneity may confer specificity to the cytoplasmic function of keratin filaments.

The biochemical relationship between individual keratins has been investigated by several groups[10–14,18]. Although the keratins of human epidermal cells grown in tissue culture are similar to those of stratum corneum of human epidermal callus with respect to their solubility properties, immunological reactivity and ability to assemble into 80-Å filaments *in vitro*, there are differences in the MWs of some of the proteins and the number of components[14,18]. In particular, the largest protein of stratum corneum (MW 63,000) is absent from the keratins of cultured human or mouse keratinocytes[14]. This high MW keratin seems to be characteristic of the stratum corneum. In another study, the keratins from two epidermal tissues of the same species,

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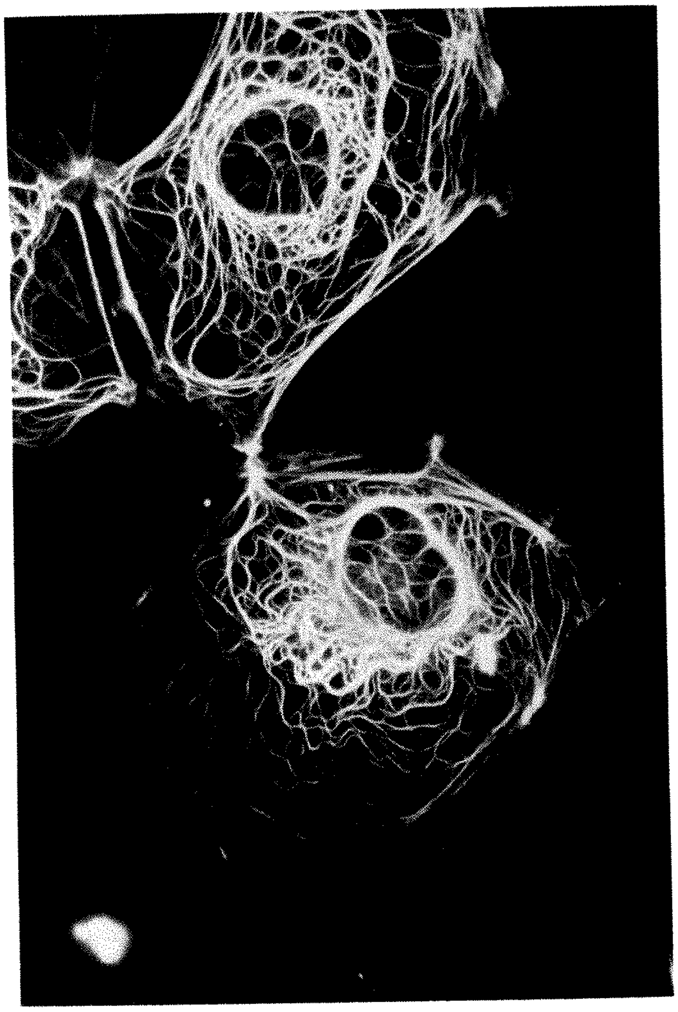

Fig. 1 The distribution of keratin filaments in epithelial cells grown in tissue culture (PtK$_2$) using indirect immunofluorescence with antibodies to keratin (courtesy of M. Osborn, K. Weber and W. W. Franke[110]).

bovine hoof and snout, were shown to be similar with respect to their MWs, amino acid compositions and percentage of helical structure[19]; however, one of the high MW (67,000) polypeptides from snout was absent from hoof keratin. In addition, the tryptic and cyanogen bromide peptides of total keratins from hoof and snout were distinctly different[19]. Thus, not only do differences exist in the keratins between different stages of epidermal cell differentiation, but also in keratins of different epidermal tissues of the same species. Furthermore, all of the keratins found in cellular extracts of cultured human epidermal cells are synthesised *in vitro* by distinct mRNA species isolated from these cells[20]. The kinetics of translation of these proteins is inconsistent with any type of precursor–product relationship within the keratin family or with the existence of precursors larger than the keratins[20]. These experiments have also clearly shown that epidermal keratins are a family of closely related but distinct proteins that are evolutionarily conserved.

Similar evidence for the existence of multiple genes was obtained first for the avian feather keratins[21,22], a family of proteins with MWs near 10,000 which assemble into 30-Å (tono) filaments and are thus quite different from epidermal keratins. By electrophoretic mobilities, amino acid composition and immunological criteria, the keratins of chicken feathers are distinct from those of scales. Differences exist as well between embryonic and adult tissues[21]. Nucleic acid hybridisation experiments have indicated that the number of genes for these low MW keratins may be 100 to 240 (ref. 22). All these results suggest that distinct sets of keratin genes are expressed during epithelial cell differentiation, in different developmental stages and in different epithelial tissues.

Desmin filaments and their role in the mechanical integration of myofibrils in muscle cells

It has been suspected that in muscle cells there is a cytoskeleton which holds the constituent myofibrils in place. The presence of structural elements transverse to a muscle fibre's long axis is suggested by the tendency for the Z disks, M lines and other sarcomeric units of neighbouring myofibrils to be in axial register across the fibre, thereby giving rise to the striking cross-striated appearance of the muscle cell[23]. Since the early days of light and electron microscopy of muscle, fibrous elements were observed to link myofibrils laterally at their Z, N and M lines and to link them to the surface membrane of the whole fibre[24–27], but only recently have they been characterised as intermediate filaments. The distribution of the filaments is characteristic of skeletal, cardiac or smooth muscle cells.

While intermediate filaments are present in both the embryonic and adult forms of all three muscle types [28–35], they are most abundant in adult smooth muscle. Here, these filaments form an interconnected network that links cytoplasmic dense bodies with membrane-bound dense plaques[30,34,35]. Morphological studies have shown that actin filaments are also inserted into these electron-dense structures[29], which can be shown to contain α-actinin and tropomyosin using immunocytochemical techniques[36]. Thus, smooth muscle dense bodies appear analogous to striated muscle Z lines.

Extractions of smooth muscle cells with high salt buffers renders contracile actin and myosin filaments soluble, leaving behind a cytoskeleton composed predominantly of intermediate filaments still attached to dense bodies[34,35]. Electrophoretic analysis indicates that this cytoskeleton consists of two major proteins: actin; and a 50,000–55,000-MW protein which we have named desmin (from the Greek noun δεσμός = link, bond)[37,38]. An identical protein has been isolated by Small and Sobieszek from mammalian smooth muscle and has been referred to as skeletin[39]. On dialysis or neutralisation, purified desmin polymerises into into a gel-like network of filaments. Reconstituted desmin filament have the same average diameter (100 Å) as smooth muscle intermediate filaments[38,39]. A small amount of actin appears to be stably associated with desmin[38,40]. The actin bound to desmin is likely to be in a conformation different from thin filament actin. It is not yet known if the interaction of desmin with actin is restricted *in vivo* in its extent and spatial location, but such an interaction might well occur at cytoplasmic or membrane-bound dense bodies, where actin and desmin filaments exist in very close proximity.

The small size of smooth muscle cells and their complicated morphology make *in situ* studies difficult, and for this reason we have used skeletal muscle for our cytological investigations of desmin. Although common in embryonic striated muscle, intermediate filaments become progressively less visible as the sarcomeres condense and become laterally registered. Intermediate filaments are rarely seen in adult muscle[41], but substantial amounts of desmin can be detected in adult striated muscle by electrophoretic and immunological methods[37,42–44]. Using immunofluorescence on longitudinal sections of myofibrils, desmin was shown to be localised in both skeletal and cardiac muscle Z lines and in regions of cell–cell juncture and apposition of the Z line with the plasma membrane (cardiac intercalated disks)[37,42,44,45]. Desmin is thus the third protein, in addition to α-actinin and actin, to be localised at the Z line using immunocytochemical techniques[45–47]. To obtain information on the spatial relationships of these three proteins within the plane of a single Z disk, it was necessary to develop a method which allowed a face-on view of Z disks by light microscopy as well as by electron microscopy. Normally when a muscle fibre is sheared, it is cleaved lengthwise, between laterally associated Z disks, to produce myofibrils, but we discovered that if most of the myosin and actin are first extracted from the fibre with high salt, the fibre's longitudinal strength is reduced and subsequent blending shears the fibre transversely between Z planes, producing honeycomb-like sheets of Z disks[48]. The generation of sheets of interconnected Z disks lends support to the idea that the Z disks within a Z plane are actually connected to one another. Electrophoretic analysis indicates that these Z planes are composed predominantly of actin and desmin (as is the case with smooth muscle dense body cytoskeletons).

Indirect immunofluorescence performed on a single Z disk sheet reveals that desmin is present at the periphery of each Z disk, forming an interconnecting network across the fibre (Fig. 2*a*). α-Actinin is localised within each disk, giving a face-on fluorescence pattern that is complementary to that of desmin (Fig. 2*b*). Actin is present throughout the Z plane, apparently co-existing with both desmin and α-actinin[48]. Thus, the Z disk is composed of two molecular domains, a peripheral one which contains at least desmin and actin, and a central one which contains at least α-actinin and actin. Do these two domains assemble at different times during myogenesis? In the early stages of myogenesis, electron microscopy[31,32,41] and immunofluorescence with desmin-specific antibodies[49] reveal that numerous desmin filaments exist scattered in the cytoplasm, not associated with the newly forming Z disks and sarcomeres. During this time the filaments can be induced to aggregate into bundles by exposing the cells to colcemid (Fig. 3)[31,42,49,50]. Later in development, desmin and intermediate filaments begin to associate with and encircle the Z disks[49] and become resistant to aggregation by colcemid[49]. Therefore, desmin and other Z disk components may undergo a 'molecular maturation' during myogenesis which allows their association.

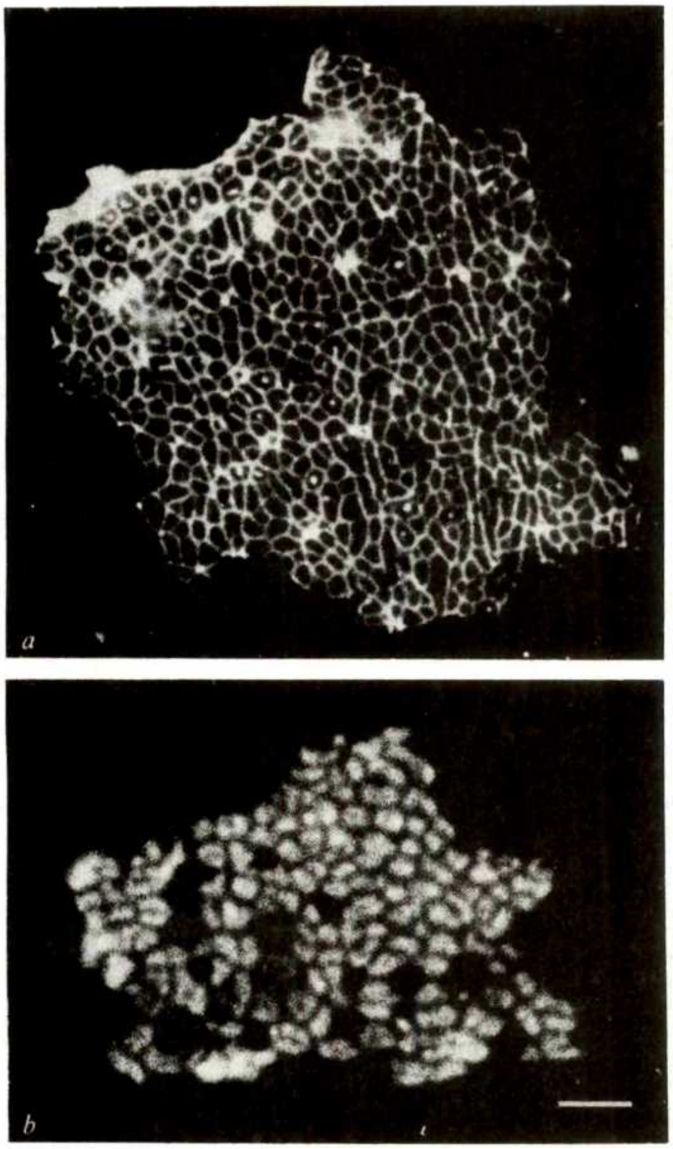

Fig. 2 *a*, The distribution of desmin at the periphery of Z disks in isolated Z disk sheets from chicken skeletal muscle. Indirect immunofluorescence using antibodies to chicken smooth muscle desmin. Vimentin shows the same distribution as desmin in these sheets (see ref. 49). *b*, The distribution of α-actinin in the interior of Z disks in isolated Z disk sheets from chicken skeletal muscle. Indirect immunofluorescence using antibodies to chicken muscle α-actinin. Scale bar, 2.5 μm.

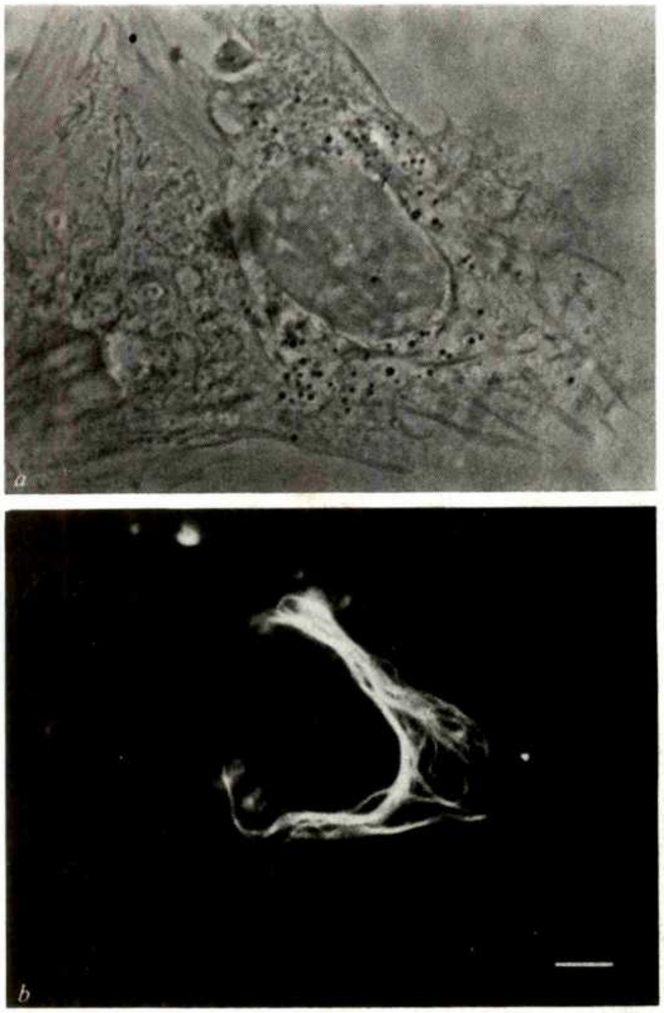

Fig. 3 The perinuclear aggregation of intermediate filaments in primary chick embryonic cardiac cells exposed to colcemid. *a*, Phase contrast image; *b*, fluorescence image. Indirect immunofluorescence using antibodies to chicken smooth muscle desmin. Similar images are obtained using antibodies to vimentin (see text). Scale bar, 10 μm.

In most types of skeletal muscle, the Z disks are encircled by the electrically excitable transverse-tubular (T) membrane. This membranous system is an extension of the plasma membrane and forms junctions (triads) with the terminal cisternae of the calcium-sequestering sarcoplasmic reticulum (SR) membrane which surrounds the rest of the myofibril[51–56]. Electron microscopy of isolated Z disk sheets (Z planes) shows membranous elements, presumably of T-system and SR origin, surrounding the individual Z disks[48]. Those same regions react strongly when the sheets are exposed to specific probes that fluoresce only in a hydrophobic environment[57]. The distribution of the membranous material in Z planes is coincident with that of desmin and complementary to that of α-actinin[57].

Linkages between adjacent myofibrils may not be restricted to the Z disk. Electron microscopy of actomyosin-depleted myofibrils demonstrates that fibrous linkages exist between adjacent myofibrils also at the M and N lines[48]. In some types of muscle the triadic junctions of the T-system are positioned at the sites of overlap of the thin and thick filaments (A–I junction)[56]; similar fibrous linkages may be involved in anchoring the triads to the periphery of the myofibril at these sites. The molecular composition of these links is presently unknown.

One seemingly puzzling observation is that in adult skeletal muscle fibres intermediate filaments are only rarely seen by conventional electron microscopic techniques. However, in certain cases, bundles of intermediate filaments have been observed surrounding skeletal muscle Z disks and extending laterally between them[41,56,58]. The presence of high concentrations of membranous material in the intermyofibrillar space may frequently obscure the presence of intermediate filament connections between adjacent Z disks, M lines and N lines[59]. Similarly, in cardiac muscle, bundles of intermediate filaments appear to surround Z disks and to connect them to the triad junctions irrespective of the position of the triad within the sarcomere (that is, at the Z line or A–I junction)[60–64]. Occasionally these filaments extend from the Z disks to the nuclear and plasma membranes[61–63]. Intermediate filaments are especially prominent in hypertrophied myocardial cells[61] or in cardiac muscle cells of animals treated with anabolic steroid hormones[64].

On the basis of these observations, we have proposed the following functions of desmin and desmin filaments in muscle cells[37,40,48,65], a schematic representation of which is shown in Fig. 4. First, desmin may mechanically integrate all contractile actions of a muscle fibre by linking individual myofibrils laterally at their Z disks and by linking Z disks to the plasma membrane and to other cytoplasmic membranous organelles. This arrangement will also ensure the maintenance of the proper alignment of cellular structures during the contraction–relaxation cycle of muscle. During the biogenesis and assembly of myofibrils, desmin may have an important role in the generation of the striated appearance of muscle by bringing the Z disks into lateral register. Second, desmin may function in the biogenesis of the T–SR membrane system. Some mechanism is required to direct the deposition of this membrane system to the periphery of the Z disk. A protein with both membrane and Z disk affinities, like desmin, could be vital in this process. As desmin binds to actin, desmin may also mediate the attachment of actin at additional membrane sites such as cardiac intercalated disks and smooth muscle dense bodies. We can further hypothesise that this transverse mechanical integrating system of desmin filaments may be an essential feature of all types of muscle regardless of their position in the evolutionary scale[44]. The proposed function of desmin indeed seems to justify its name and provides the first well documented cytoskeletal function for intermediate filaments.

Vimentin filaments

A variety of cells of mesenchymal or non-mesenchymal origin contain vimentin filaments (see Table 1), so called because immunofluorescence studies with antibodies to the major subunit of these filaments reveal a wavy (Latin *vimentus*) cytoplasmic filamentous system (Fig. 5)[6,73].

Vimentin was first characterised as a 52,000-MW, major subunit of intermediate filaments in chick embryo fibroblasts[66]. The wide cross-reactivity of vimentin antibodies with cells of mammalian, avian or amphibian origin indicates that this protein is conserved in evolution[6,67–69].

Table 1 Presence of intermediate filament subunits in different cell types

Cell type	Vimentin (MW 52,000)	Desmin (MW 50,000)	Keratins (MW 40,000–65,000)	Glial filaments (GFA) (MW 51,000)	Neurofilaments (MW 200,000, 150,000, 68,000)
Adult muscle:					
Skeletal, cardiac, smooth	+	+	−	−	−
Myotubes	+	+	−	−	−
Myoblasts	+	+	−	−	−
Fibroblasts	+	+	−	−	−
3T3	+	+	−	−	−
SV40-3T3	+	ND	−	−	−
BHK–21	+	+	−	−	−
NIL-8	+	−	−	−	−
Sarcoma 180	+	ND	−	−	−
Schwann cells	+	+	−	−	−
Neuroblastoma	+	ND	−	−	ND
Pigment cells	+	−	−	−	−
Skin melanocytes	+	ND	−	−	−
Chondroblasts	+	−	−	−	−
Endothelial cells	+	−	−	−	−
PNS, CNS neurones	−	−	−	−	+
Glial cells:					
Astrocytes	+	−	−	+	−
Oligodendrocytes					
Microglia					
Ependymal cells					
Glioma C6					
Epithelial cells:					
HeLa	+	−	+	−	−
Rat kangaroo PtK$_1$, PtK$_2$	+	−	+	−	−
Epidermal cells	−	−	+	−	−
Cultured keratinocytes	+	−	+	−	−
Stratified squamous epithelia	−	−	+	−	−
Epidermal appendages	−	−	+	−	−
Hassal's corpuscles of the thymus	−	−	+	−	−
All other epithelial cells	+	−	+	−	−
Exceptions:					
Epithelia of iris and lens	ND	−	−	−	−
Glomerular and tubular cells of kidney	ND	−	−	−	−
Liver hepatocytes	+	ND	+	−	−
Hepatoma cells	+	ND	−	−	−
Pancreas	ND	ND	−	−	−

Compiled from data in refs 3, 4, 5–7, 37, 38, 42–44, 67–69, 73, 86, 95–102, 108, 109. ND, not determined.

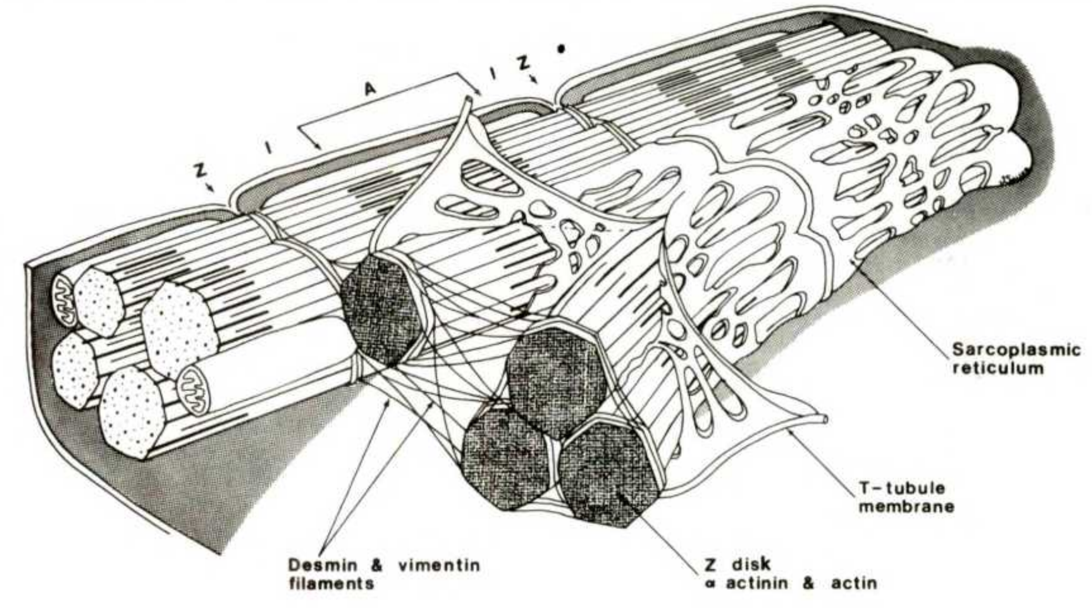

Fig. 4 Schematic representation of the distribution of desmin, vimentin, α-actinin, actin and membranous organelles in relationship to chicken skeletal myofibril Z disks.

One of the most interesting and characteristic properties of vimentin-containing intermediate filaments is their aggregation into perinuclear filamentous bundles in the form of a cap or a ring in cells exposed to colcemid[70–73], a finding which originally prompted the erroneous belief that these filaments were degradation products of microtubules. Similar structures exist transiently during normal cell adhesion and spreading onto a substrate[74,75] or in non-motile tumour cells[76]. Isolated filament caps contain two predominant polypeptides in approximately equimolar amounts, one with a MW of 52,000–55,000 which corresponds to vimentin in other cell types, and the other with a MW of 50,000–54,000 which, as discussed below, corresponds to muscle desmin. Vimentin and desmin can be purified from the birefringent caps by repeated cycles of disassembly–reassembly, induced by lowering and raising the ionic strength of the extracting solutions[75,77]. Reassembled filaments have an average diameter of 100 Å; it is not known whether vimentin and desmin copolymerise into a single filament or whether they polymerise into distinct filaments with common properties. Vimentin has now been identified in a variety of cell types, quite frequently in co-existence with the major subunit of some of the other classes of intermediate filaments (see below).

Vimentin filaments are very insoluble, suggesting that they have a structural function in the cytoplasm. They frequently terminate at both the nuclear membrane and the adhesion plaques of detergent-extracted cytoskeletons prepared from cultured fibroblasts[78,79] or nucleated chicken red blood cells[80]. In normal conditions (in the absence of colcemid), there is always a certain perinuclear concentration of 100-Å filaments in the cell lines studied[79]. Enucleation of cells by cytochalasin B produces karyoplasts surrounded by intermediate filaments and cytoplasts in which intermediate filaments remain situated in the central region of the cytoplasm[79]. Together these results strongly suggest that vimentin is tightly associated with the cell nucleus, possibily providing it with mechanical support and constraining it in a specific place in the cell[78–80]. In this respect, several other observations have also been made of the association of intermediate filaments with centrioles *in situ*[75,81].

The fate of vimentin filaments during the cell cycle appears to vary from one cell type to another. In BHK-21 cells the filaments disaggregate during prometaphase and reappear as filamentous caps in the perinuclear area during the spreading of the two daughter cells[75]. However, in most other cell types (NIL-8, endothelial cells), the filaments appear to persist in a polymerised form throughout mitosis[72,73,82]. In vascular endothelial cells, the interphase cells contain a ring of intermediate filaments that encircles the nucleus and is maintained in a plane parallel to the substrate[72,82]. During prophase and metaphase the cells round up and the filament ring becomes wavy, although it still exists as a closed structure. During anaphase the ring elongates into a rectangle that contains the spindle apparatus and chromosomes. In late telophase, cytokinesis cleaves the filaments into crescents at the site of the contractile ring, which then close into rings in the daughter cells[82] (see Fig. 6).

Neurofilaments and glial filaments

Neurofilaments (average diameter 100 Å) and microtubules (250 Å diameter) comprise the main structural elements of neuronal axons, dendrites and perikarya. The neurofilaments can be either randomly or uniformly dispersed and appear to be linked to each other through wispy sidearm appendages. Glial filaments are found randomly dispersed in the cytoplasm of astrocytes and other types of glial cells; in contrast to the neurofilaments, glial filaments are more closely packed (average diameter 80 Å), are less parallel in their orientation and lack sidearm appendages[83].

There has been prolonged controversy and confusion over the subunit composition of neurofilaments and glial filaments. The first intact intermediate filaments to be isolated[84] were derived from myelinated nerve fibres and were therefore assumed to be characteristic neurofilaments with a 51,000-MW protein derived therefrom being the neurofilament protein. This assumption was erroneous. It has since been clearly demonstrated that these filaments were predominantly glial filaments with a 51,000-MW glial fibrillary acidic (GFA) protein as their major subunit.

When neurofilaments are purified from the peripheral nervous system (PNS), which is relatively free of glial cells, no protein with a MW of 51,000 is detected[85,86]. Instead, rat peripheral nerve, rat spinal cord and rabbit intradural spinal nerve roots[85–88] each contain three major polypeptides with MWs of 200,000, 150,000 and 68,000. In contrast, squid neurofilaments are composed of only two major polypeptides with MWs of 200,000 and 60,000 (refs 89–91), while those from the giant axon of the marine worm, *Myxicola infudibulus*, are composed of two polypeptides with MWs of 160,000 and 150,000 (refs 89, 92). In spite of these differences, however, one of the characteristic properties of both vertebrate and invertebrate neurofilaments is their extreme susceptibility to proteolysis in the presence of Ca^{2+} ions[92,93].

The biochemical evidence distinguishing neurofilaments from glial filaments has been supported by immunological studies with antibodies prepared with and specific for the purified subunit proteins of neurofilaments and glial filaments. Antibodies raised to the 68,000-MW components of mammalian peripheral nerve react in immunofluorescence with axons and

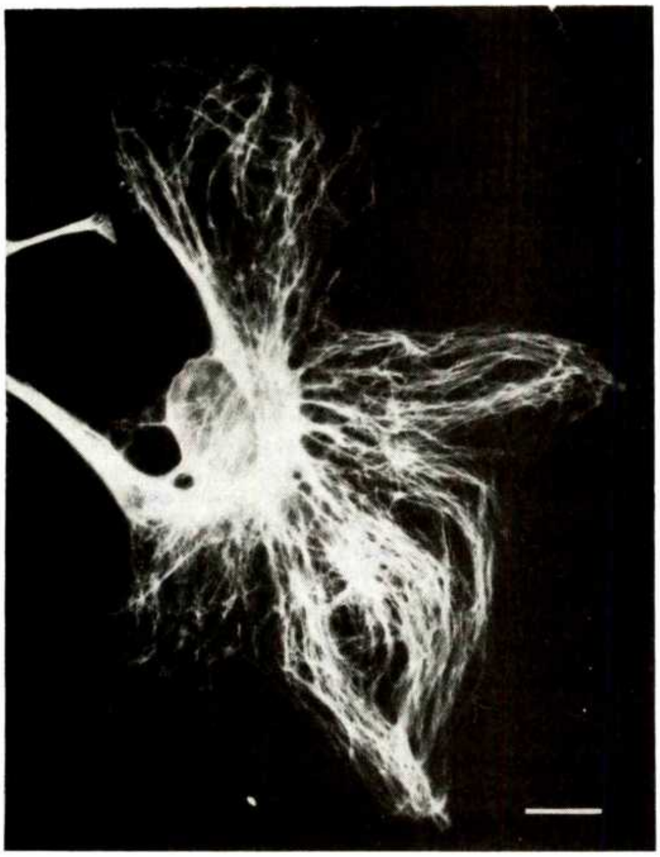

Fig. 5 The distribution of intermediate filaments in a normal chick embryonic fibroblast using antibodies to chicken vimentin in indirect immunofluorescence (courtesy of B. L. Granger). Scale bar, 10 μm.

neurones throughout mammalian PNS and central nervous system (CNS) but do not react with glial cells[86,94,95]. Similarly, antibodies to GFA react in immunofluorescence only with glial cells or cells of glial origin and do not react with neurones or their processes[86,96,97] (for a review on GFA and its properties see ref. 98). Antibodies to GFA do not react with muscle cells, myofibrils, or fibroblasts, indicating it is immunologically distinguishable from vimentin and desmin[44]. Conclusive demonstration that GFA is the major component of glial filaments stems from the observations that (1) GFA is the major component of purified glial filaments[99]; (2) purified GFA will assemble *in vitro* into filaments with an average diameter of 100 Å (ref. 98); and (3) antibodies to GFA will react with glial 100-Å filaments *in situ* using the immunoperoxidase method[96].

While the function of glial filaments is presently undetermined, neurofilaments appear to function as a three-dimensional structural lattice that provides tensile strength to the axons. This idea stems from the observation that the extruded axoplasm from *Myxicola* is a highly structured gel consisting almost exclusively of neurofilaments[91,92]. Exposure of this gel to Ca^{2+} results in the degradation of neurofilaments and the dissolution of the gel.

Co-existence of different intermediate filament subunits in the same cell

A number of cells have recently been shown to possess the subunits of at least two distinct intermediate filament types, sometimes in clearly distinguishable filamentous systems. For example, PtK_2 and HeLa cells have abundant keratin filaments in their cytoplasm and contain vimentin in the perinuclear aggregates of intermediate filaments experimentally induced by colcemid[6,7]. Both polypeptides can also be demonstrated in these cells by electrophoretic analysis of their filamentous detergent insoluble cytoskeletons[6,7].

Similarly, two-dimensional electrophoresis of the cytoskeletons from fibroblast-free cultures of chicken embryonic muscle, chicken embryo fibroblasts, 3T3 cells and BHK-21 cells shows that all these contain not only vimentin but also a second polypeptide which is highly homologous to muscle desmin[100,101] and can be detected by immunofluorescence in colcemid-induced perinuclear rings of intermediate filaments[100]. In skeletal myotubes and isolated myofibrils, the distribution of desmin and vimentin coincides[49].

Recent immunofluorescent experiments also suggest that glial cells (glioma cells and astrocytes) contain vimentin in addition to the glial-specific filament subunit (GFA)[6,68,69,102]. In cultured astrocytes, vimentin and glial filaments can be distinguished by their characteristic immunofluorescence patterns. The possible presence of vimentin in purified glial filaments and the natural occurrence of autoantibodies to vimentin may explain the wide cross-reactivity observed in earlier studies with antibodies to GFA[103].

These observations demonstrate that desmin and vimentin can co-exist in muscle and non-muscle cells; similarly, vimentin and certain keratins can co-exist in some epithelial cell types, and vimentin and GFA can co-exist in glial cells. While vimentin and keratin filaments can be distinguished easily by electron microscopy and their responses to colcemid, this is not the case with vimentin and desmin filaments. Both desmin and vimentin filaments appear morphologically (that is, by transmission electron microscopy) very similar and aggregate into perinuclear rings in cells exposed to colcemid (see Fig. 2*b*). As desmin and vimentin from BHK-21 cells co-purify through cycles of polymerisation–depolymerisation (see above), it will be intriguing to find out whether the two polypeptides can co-polymerise into one and the same filament, as is the case with the keratins, or whether they polymerise into distinct filaments. Immunoelectron microscopy with desmin and vimentin antibodies should answer these questions. At present, the function of desmin in non-muscle cells and of vimentin in muscle cells is unknown, but by analogy to muscle cells, desmin may be involved in the adhesion of actin filaments to the plasma membrane.

Phosphorylation of intermediate filament subunits

All intermediate filament subunits examined to date seem to be phosphorylated *in vivo* and can be phosphorylated *in vitro*. For example, phosphorylation of the 200,000 but not the 60,000-MW polypeptide from squid axoplasm occurs after intracellular injection of $[\gamma\text{-}^{32}P]ATP$ into giant axons or addition of $[\gamma\text{-}^{32}P]ATP$ to extruded axoplasm[90]. In contrast, both *Myxicola* neurofilament polypeptides are phosphorylated *in vivo*, probably by a soluble kinase which is independent of Ca^{2+} cyclic AMP or cyclic GMP for its activity[104]. A portion of this activity remains associated with neurofilaments even after extensive purification of the filaments[104]. Similarly, all three polypeptides of the mammalian neurofilament triplet[104] are phosphorylated.

A non-phosphorylated and several phosphorylated variants of desmin and vimentin are present in assembled structures (Z disks, dense body attached and cytoplasmic intermediate filaments) in muscle[105] and non-muscle cells[100,105,106] while some of the keratin polypeptides are also multiply phosphorylated[14].

Three major activities can be identified in muscle extracts that phosphorylate purified smooth muscle desmin or desmin and vimentin in fibroblastic filamentous cytoskeletons. Two of the activities co-purify with the muscle cyclic AMP-dependent protein kinases and are inhibited by the cyclic AMP-dependent protein kinase inhibitor protein[107]. The other activity seems to be cyclic AMP independent[107].

The role of phosphorylation in the regulation of assembly or any other function of intermediate filaments is at present unknown. However, as assembled intermediate filaments are always phosphorylated, phosphorylation may be involved in determining the state of polymerisation or aggregation of these structures.

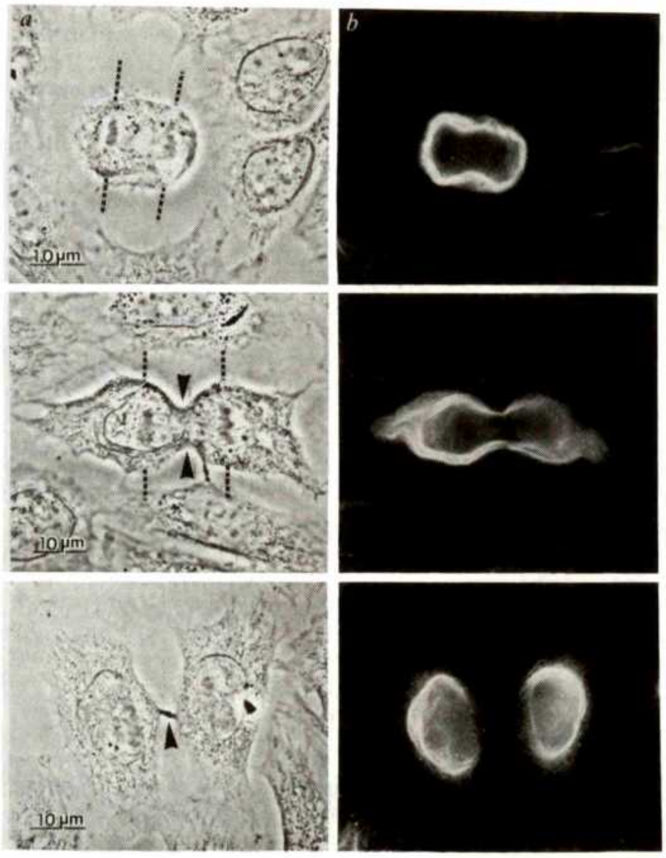

Fig. 6 Endothelial cells during mitosis. Phase (*a*) and immunofluorescent (*b*) micrographs using antibodies to vimentin. Scale bars, 10 μm. Top, anaphase. The dotted lines indicate the position of the chromosomes. Middle, telophase. The contractile ring, indicated by arrowheads in the phase micrograph, begins to pinch the intermediate filaments observed by immunofluorescence. Bottom, late telophase. Cytokinesis has now completely cleaved the ring into two symmetrical crescents that enter the daughter cells. The arrow points to the midbody (from S. Blose, ref. 82).

Prospects for the future

Now that the biochemical and immunological taxonomy of the various intermediate filaments is nearly complete, certain intriguing biological questions arise. The majority of cytoplasmic microtubules and actin filaments emanate from or terminate at one or more nucleation or organisation sites. No such sites have yet been identified for intermediate filaments, but they would have to exist to provide directionality of growth and three-dimensional organisation to these filaments. Keratin filaments are frequently found in association with desmosomes, desmin filaments with substructures of intercalated disks and vimentin filaments with the nuclear membrane; perhaps these structures can function as nucleation sites for the growth of the respective filaments. In general, intermediate filaments are insoluble in physiological buffers, but highly susceptible to proteolysis, especially in the presence of Ca^{2+}. As these filaments exist mainly in the cytoplasm in a full polymeric state, perhaps their disassembly is regulated by a proteolytic mechanism rather than by a polymerisation–depolymerisation equilibrium as is the case with microtubules. Such a proteolytic mechanism would have an important regulatory role in shape changes that occur during locomotion and cell division.

The co-existence of desmin and vimentin is interesting and it will be important to determine whether the two proteins copolymerise into one and the same filament or co-exist in distinct independent filaments. Skeletal muscle differentiation emerges as a very interesting system to elucidate the proposed function of desmin filaments, the molecular details of the interaction of these filaments with actin and the various types of membranous organelles and the molecular events that lead to their ultimate association with the Z disks. The high degree of keratin polymorphism and the changes in their expression during epidermal differentiation make epidermal differentiation *in vitro* an attractive model system to study the developmental expression of the keratin genes. Finally, although intermediate filaments can be distinguished by immunological methods and peptide mapping, they share a number of properties. To reconcile their morphological similarity and chemical heterogeneity, it is possible to imagine that the subunits of the intermediate filaments are analogous to IgG molecules, namely, that they are composed of a constant and a variable domain. The constant domain might be responsible for the conserved polymerisation of the protein subunits into filaments with an average diameter of 100 Å and their aggregation into filamentous bundles in cells exposed to colcemid. The variable domain might confer on the molecule functional specificity for the cell of which it is a part. Amino acid sequencing data will reveal the validity of this proposition.

Intermediate filaments have emerged as a polymorphic class of cytoplasmic filaments distinct from microtubules and actin filaments. All their biochemical and morphological properties so far indicate that they are involved in mechanically integrating the various components of the cytoplasmic space. As such they should be capable of multiple interactions with cytoplasmic structures, might be expected to have a crucial role in regulation of cell shape and may be influenced by the metabolic state and response to injury or malfunction of any cell in which they are contained.

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ARTICLES

^{187}Re–^{187}Os systematics in meteorites: early chronology of the Solar System and age of the Galaxy

Jean-Marc Luck, Jean-Louis Birck & Claude-Jean Allegre

Laboratoire de Géochimie et Cosmochimie LA 196, Institut de Physique du Globe et Département des Sciences de la Terre,
Université Pierre et Marie Curie, Paris VI et VII, 4 Place Jussieu, 75230 Paris Cedex 05, France

Using isotope dilution mass spectrometry, we have determined the $^{187}Re/^{186}Os$, $^{187}Os/^{186}Os$ ratios of five iron meteorites of various groups, and one ordinary chondrite. These results show that iron meteorites and ordinary chondrites were formed within a short time. We determine the ^{187}Re decay constant as $1.62 \pm 0.03\ 10^{-11}\ yr^{-1}$ and the $^{187}Os/^{186}Os$ isotopic ratio at the origin of the Solar System as 0.805 ± 0.011. These values are used to compute limits for the age of the Galaxy between 13,300 and 22,400 Myr.

^{187}Re is one of the long-lived radioactive nuclides with a decay constant (λ) of the order of 10^{-11} yr^{-1}. It produces ^{187}Os by (β^-) decay. Therefore, like ^{87}Rb–^{87}Sr or ^{40}K–^{40}Ar, the ^{187}Re–^{187}Os system is a potential cosmochronometer[1]. Some peculiar properties of the Re–Os system make it particularly attractive for dating the early formation of the Solar System and for estimating the age of the Galaxy.

(1) Re and Os are refractory siderophile elements that concentrate in the metal and sulphide phases of meteorites[2]. These phases are the dominant material not only in iron meteorites, but also in pallasites and mesosiderites. With regard to ordinary and enstatite chondrites, Re and Os are also mainly contained in the metal phase. Thus Re–Os measurements on this metal phase will give the absolute age of different types of meteorites.

For iron meteorites Re/Os fractionation, being presumably related to the fractional crystallisation of metal in the core of their parent body[3], the eventual isochron is expected to date the formation of irons, that is, the differentiation of parent bodies.

In this way, it would be possible to propose a relative chronology of the early history of the Solar System by comparing the respective ages of, for example, irons and chondrites. Note that in the case of irons this chronometer will lead to an accurate dating, a controversial subject because no other reliable absolute chronometer can be applied to the metal itself: the Rb/Sr is very sensitive to secondary perturbation events[4], Pb isotopic composition is primordial in iron meteorites[5], and K–Ar has a limited use because of spallation phenomena.

(2) ^{187}Re has a half life of the order of magnitude of the age of the Galaxy[6,7] and is, therefore, potentially a good way of obtaining precise estimates for this age. If ^{187}Re is produced by (r) processes, ^{187}Os and ^{186}Os are produced by (s) processes only and hence their production rates are not submitted to the uncertainty of the (r) process.

Besides these advantages, the ^{187}Re–^{187}Os system contains some basic difficulties: first, we do not know the decay constant exactly

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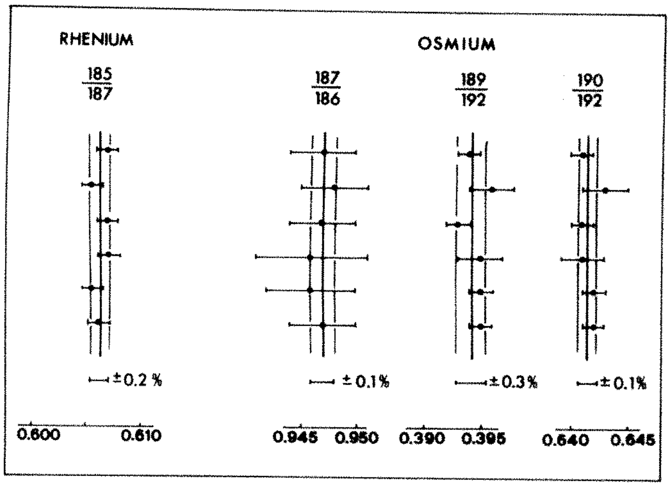

Fig. 1 Reproducibility of ion-probe measurements of standard isotopic compositions of Re and Os.

because its direct measurement by counting methods in the laboratory is very difficult[8]; second, the content of Re and Os in meteorites is quite low (3–0.3 p.p.m.) and the precise isotopic analysis of osmium isotopes is quite difficult at this level.

These difficulties explain why the only serious attempt to use the ^{187}Re–^{187}Os chronometer in cosmochronology was done by Herr *et al.*[1] and Hirt *et al.*[9] in the early sixties. The German–Swiss group has approached the above difficulties by determining the rhenium decay constant using terrestial molybdenites the age of which was approximately known[10]. They obtained $1.61 \pm 0.19 \times 10^{-11}$ yr^{-1}. To overcome the second problem they used gas source mass spectrometry but their technique was neither very sensitive nor precise. Using their previously determined decay constant, they obtain for iron meteorite samples from several groups an age of 4,000 Myr.

We have used a more refined technique for Re and Os measurements, considered a new set of iron meteorites plus one chondrite, and recalculated the age of the Galaxy.

Experimental technique

We have used, both for Re and Os, isotope dilution mass spectrometry, using ^{185}Re and ^{190}Os spikes prepared by Oak Ridge National Laboratories. After dissolution and spiking Re and Os are separated chemically, then loaded on an aluminium disk and run separately in an ion-sputtering mass spectrometer. The separation between Os and Re is absolutely crucial because they interfere at mass 187—the mass of interest.

Chemistry: The details of the technique will be published elsewhere and only the principles are described here.

Metal phases are dissolved in 6M H_2SO_4 and at the same time ^{185}Re and ^{190}Os spikes are added. Oxidation is carried out through an excess of CrO_3 and osmium is distilled at 110 °C as OsO_4 into a cooled water trap. After transforming the oxide to bromide then to chloride, Os is loaded on an anion-exchange column for purification. It is eluted with 4M HNO_3 and then loaded on a 99.99% pure aluminium disk.

Re is extracted from the distillation residue by tribenzylamine in chloroform and then back extracted with ammonia into aqueous solution. It is loaded on an anion-exchange column, eluted with 8M HNO_3 and then loaded on a pure aluminium disk.

Mass spectrometry: The Cameca SMI300 ion probe was used as a sputtering mass spectrometer. The sputtering is done by a negative oxygen ion beam. The target is the Os or Re deposit on the aluminium disk. The machine which was largely modified for our experiments has been described elsewhere[11]. It is equipped with an energy filtering system through an electrostatic analyser nd a programmable magnetic field and digital output. Energy iltering eliminates most of the possible interfering species ncluding hydrocarbons.

Performances: The total blanks of our chemistry are: for Re < 3ng; for Os < 5ng. At such a low level the measurements are inprecise due to small interferences; we have, therefore, only estimated the possible upper limits. In fact, blanks are probably much lower. The reproducibility of isotopic ratio measurements in the mass spectrometer is ±0.2% for Re, ±0.1% for Os. The standard runs are illustrated in Fig. 1. A duplicate analysis has been done on the iron meteorite Tlacopetec to estimate whether isotope dilution was achieved:

Experiment 1 Re = 3.69 ± 0.03 p.p.m.
Os = 57.8 ± 0.3 p.p.m.
Experiment 2 Re = 3.29 ± 0.02 p.p.m.
Os = 57.7 ± 0.8 p.p.m.

These results show an extremely good reproducibility.

Meteorite chronology

As established by Hirt *et al.*[10] we can write the chronometric equation for a closed system using ^{186}Os as reference isotope.

$$({}^{187}\mathrm{Os}/{}^{186}\mathrm{Os})_{\mathrm{Today}} = ({}^{187}\mathrm{Os}/{}^{186}\mathrm{Os})_{T_0} + ({}^{187}\mathrm{Re}/{}^{186}\mathrm{Os})_{\mathrm{Today}}(e^{\lambda T_0} - 1)$$

where T_0 is the age at the closure of the system.

This equation is then used to compare ^{187}Os/^{186}Os with ^{187}Re/^{186}Os to determine the age of the system, the initial isotopic ratio of osmium, and at the same time to check if the subsystems are cogenetic and closed. We have thus analysed several iron meteorites of different classes: Canyon Diablo (IB), Coahuila (IIA), Henbury (IIIA), Casas Grandes (IIIA), Tlacotpec (IVB) and one ordinary chondrite, St Séverin (LL6). For each meteorite the iron part has been taken (separated in the case of St Séverin) and treated as described above. The results, given in Table 1 and Fig. 2, show a wide range of Re/Os values.

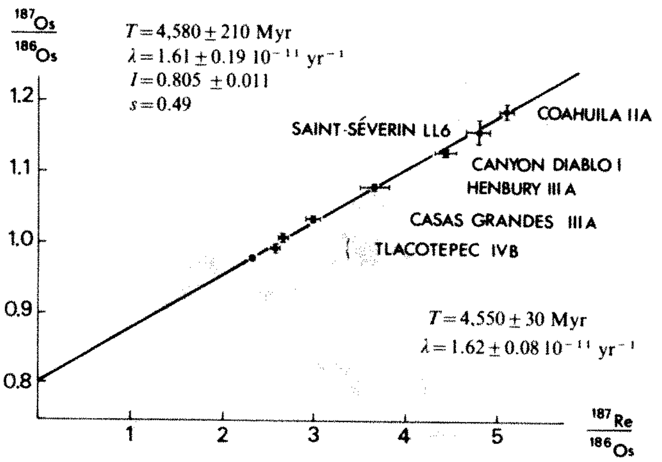

Fig. 2 ^{187}Re–^{187}Os evolution diagrams for five different iron meteorites from four different groups and one chondrite. *s* is the reduced χ^2 value and indicates the excellent fit to a straight line.

From both our isotopic measurements and from expected ^{187}Os/^{186}Os ratios for irons of various classes[2], it seems that the Re/Os ratio and, hence the radiogenicity of osmium, could depend on the class to which the iron belongs. That is, in general, meteorites from class I are expected to be more radiogenic than those from class IIIA, and less than that from class IIA. This slight trend could be related to the oxidation state of irons. However, the same data do not presently suggest any trend within a given group. In this case, the Re and Os fractionation might involve another factor besides the degree of fractional crystallisation of the metal phase. More measurements are needed to confirm these results.

Regarding the measurements made on the iron meteorites, all points fall remarkably on a single line defining an extremely good isochron ($s = 0.49$). The slope is 0.0765 ± 0.0035 and the initial ratio $^{187}Os/^{186}Os = 0.805 \pm 0.011$ (2σ errors). These two results differ substantially from the results of Hirt *et al.*[9] for the slope, as well as for the initial ratio and the precision (respectively 0.066 ± 0.013 and 0.83 ± 0.06). However, this difference is the normal difference which can be expected from almost 20 years of analytical progresses into the field: note that all our results are within the error limits given by the German–Swiss group. Using the decay constant $\lambda_{Re} = 1.61 \times 10^{-11}\ yr^{-1}$, we obtain an age of 4,580 ± 210 Myr.

Several conclusions can be drawn from these results.

First, within the accuracy of our measurements, the iron meteorites of several groups were formed with a short time interval with ordinary chondrites. Such an unequivocal result has not been clearly established before, except by I–Xe chronology[12,13]. The Rb–Sr approach of the Caltech group gave various ages from 4,700 to 3,800 Myr and no clear answer to this question[14]. However, all the precedent results dated the silicate inclusions, not the iron itself. Our results show that if iron meteorites come from differentiated bodies, such a differentiation occurred within a period of about 400 Myr in their respective parent bodies.

Second, the time interval between these events and the well determined formation of ordinary chondrites is very short—say <200 Myr; this value is obtained from the extreme ages for St Séverin (error limits) compared with the 4,580 Myr age for iron meteorites. Indeed as most, if not all, of Re and Os from chondrites is contained in their metallic part[15], the determination for Saint Séverin can be considered as that for the whole rock of this unperturbed ancient meteorite[16]. In other words, the differentiation of planetary bodies is a very early event in the Solar System. Such an early differentiation is consistent with the existence of early magmatic activity in the Solar System as determined on the Juvinas basaltic achondrite[17].

From our results we can deduce a decay constant for the ^{187}Re. The recent results from our group clearly establish the age of formation of basaltic achondrites[17,18], H chondrites[19], enstatite chondrites[20], and LL chondrites[16,19] both by Rb–Sr and U–Pb methods[21] in the range of 4,550 ± 30 Myr. Such results are also in good agreement with the data obtained by Turner *et al.*[22] using the ^{39}Ar–^{40}Ar method, and by Lugmair using the Sm–Nd chronometer[28]. Taking the age as 4,550 ± 30 Myr, we obtain $\lambda_{Re} = 1.62 \pm 0.08\ 10^{-11}\ yr^{-1}$. Note that such a value is extremely close to the previous value of $1.61\ 10^{-11}\ yr^{-1}$ determined by Hirt *et al.*[10] on terrestial molybdenite samples. We stress again the remarkable results obtained by these authors 20 years ago.

Age of the Galaxy

We will use the values of λ_{Re} and ($^{187}Os/^{186}Os$) initial ratio to determine the so-called age of the Galaxy following at first an early approach by Clayton[6].

^{186}Os and ^{187}Os isotopes are created by (s) nucleosynthetic processes. In addition, ^{187}Os contains a radiogenic contribution from ^{187}Re. On the other hand, ^{187}Re is created by (r) process. Therefore

$$^{186}Os = {}^{186}Os(s)$$

$$^{187}Os = {}^{187}Os(s) + {}^{187}Os^*$$

Where $^{187}Os^*$ is the radiogenic contribution. On the other hand,

$$\frac{d^{187}Os^*}{dt} = \lambda_{Re} \times {}^{187}Re$$

and

$$\frac{d^{187}Re}{dt} = A(t) - \lambda_{Re}{}^{187}Re$$

where $A(t)$ is the (r) process production rate for ^{187}Re.

Following Clayton[6], we can take $A(t)$ in the form of $A_0 e^{-\beta t}$. When β is large, one obtains the limiting case of sudden nucleosynthesis. When β is small one obtains a steady-state nucleosynthesis.

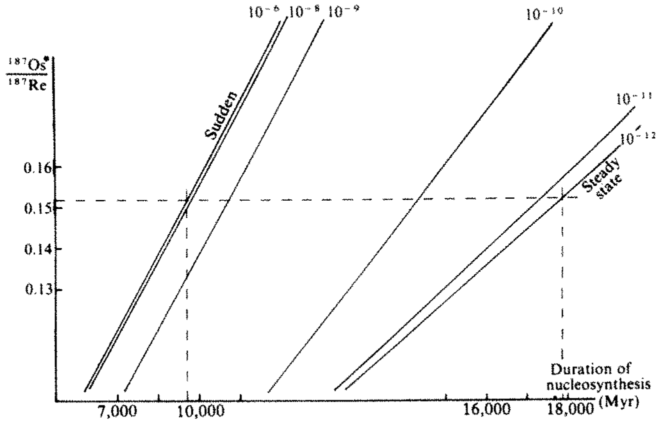

Fig. 3 Variation of the $^{187}Os^*/^{187}Re$, at the formation of the Solar System, with respect to the duration of nucleosynthesis, for different time constants (β) of the nucleosynthesis production rates.

The integration of the above differential equations give

$$\frac{^{187}Os^*}{^{187}Re} = \frac{\lambda/\beta(1-e^{-\beta\Delta t}) - (1-e^{-\lambda\Delta t})}{(e^{-\beta\Delta t} - e^{-\lambda\Delta t})} = \psi(\Delta t)$$

at the formation of the Solar System, when the solar nebula became isolated from the Galaxy. Δt is the time between starting of nucleosynthesis and meteorite formation, that is, the age of the Galaxy before the formation of the Solar System. Note that A_0 does not play any part in this equation which is a remarkable property of parent–daughter cosmochronology.

Taking as usual ^{186}Os as the reference, we obtain

$$\frac{^{187}Os^*}{^{186}Os} \Big/ \frac{^{187}Re}{^{186}Os} = \psi(\Delta t)$$

But on (s) process we know that $^{187}Os_{(s)}\sigma_{187} \sim {}^{186}Os_{(s)}\sigma_{186}$ where σ_{187} is the neutron capture cross-section of ^{187}Os.

$$\frac{^{187}Os^*}{^{186}Os} = \frac{^{187}Os}{^{186}Os_{total}} - \frac{\sigma_{186}}{\sigma_{187}} \cdot f$$

$$\frac{^{187}Re}{^{186}Os} = \left(\frac{Re}{Os}\right)_{atomic} \cdot \phi$$

where ϕ is the abundance of ^{187}Re in Re over the abundance of ^{186}Os in Os. The cross-sections (Maxwell averaged) were recently measured by Browne and Berman[23] for $kT = 30$ keV.

$$\sigma_{186}/\sigma_{187} = 0.39 \pm 0.03$$

f is a correction factor which takes into account the dependance of the cross-section on the temperature and the possible excited state of the atoms in stars. Woosley *et al.* (in ref. 7) give $f = 0.82$. The (Re/Os) ratio in carbonaceous chondrites which are supposed to represent the best estimate of the Solar System average value is 0.081 (ref. 24). The fact that this ratio does not vary too much between different meteorite classes[15,25,26], and the Earth[27] indicates that this value is probably quite constant cosmochemically speaking. From our measurements, we calculate $\phi = 40.88$, then $^{187}Re/^{186}Os = 3.20$, and $^{187}Os^*/^{187}Re = 0.152$.

Table 1 $^{187}Re/^{186}Os$ and $^{187}Os/^{186}Os$ ratio for iron meteorites and the magnetic separate from St Séverin

Sample		Sample weight (mg)	Re (p.p.m.)	Os (p.p.m.)	$^{187}Re/^{186}Os$	$^{187}Os/^{186}Os$
Iron meteorites:						
Tlacotepec (IVB)	no. 1	187.3	3.28 (3)	57.0 (4)	2.32 (3)	0.980 (4)
	no. 2	264.7	3.69 (3)	57.8 (7)	2.56 (5)	0.994 (6)
	no. 3	253.6	3.69 (3)	56.0 (6)	2.65 (5)	1.008 (6)
Canyon Diablo	IA	4075.0	0.236 (1)	2.12 (2)	4.47 (5)	1.122 (8)
Coahuila	IIA	411.9	1.40 (1)	11.11 (6)	5.06 (7)	1.187 (11)
Casas Grandes	IIIA	2446.9	0.731 (6)	9.87 (14)	2.98 (7)	1.035 (5)
Henbury	IIIA	472.0	1.53 (5)	16.9 (2)	3.63 (16)	1.080 (4)
Chondrite:						
St Séverin magnetic separate	LL6	636.8	0.939 (8)	8.00 (18)	4.72 (16)	1.164 (15)

The numbers in parentheses are the errors on the results, expressed in last digits: for example, 3.28(3) is equivalent to 3.23 ± 0.03 and 3.63(16) is equivalent to 3.63 ± 0.16.

We can then compute for these different values of (β) the curves showing the variation of $^{187}Os^*/^{187}Re$ with Δt (duration of nucleosynthesis (Fig. 3). Choosing for β the extreme values

10^{-6} yr^{-1} for sudden nucleosynthesis

10^{-12} yr^{-1} for steady state nucleosynthesis

we obtain for $^{187}Os^*/^{187}Re = 0.152$ two intercepts that give us an interval for the duration of nucleosynthesis: $8{,}700 < \Delta t < 17{,}800$ Myr and hence the age of the Galaxy is $13{,}300 < T < 22{,}400$ Myr. Note that a ±10% variation on $^{187}Os^*/^{187}Re$ would lead to a ±8% variation on T. Such an uncertainty might come from the cosmic Re/Os ratio or from the factor f or from the neutron capture cross-section ratio.

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Bursts of aftershocks, long-term precursors of strong earthquakes

V. I. Keilis-Borok*, L. Knopoff† & I. M. Rotvain*

* Institute of the Physics of the Earth, Academy of Sciences, Moscow, USSR
† Institute of Geophysics and Planetary Physics, University of California, Los Angeles, California 90024

Earthquakes that are followed within a short time by abnormally large numbers of aftershocks are hypothesised to be long-term precursors of stronger earthquakes. In a test of the hypothesis, 18 out of 23 strong earthquakes, in five regions worldwide, were predicted retrospectively. In comparison with a random model the precursory pattern occurs at a satisfactorily high confidence level.

THE hope that patterns of seismicity exist that are premonitory to strong earthquakes relies on the fact that the occurrence of earthquakes is controlled by the distribution of stresses and strengths within the Earth and that each earthquake in turn changes these fields. At present we cannot anticipate what such patterns should be, because the theoretical basis is inadequate. One reason for this is the absence of firmly established empirical regularities in seismicity which would provide a factual basis for theoretical studies of earthquake occurrence.

Searches for premonitory patterns of seismicity have attracted much attention, as these may possibly be established by the analysis of earthquake catalogues which are the longest record of the relevant observations. With regard to precursors of large earthquakes, there have been many purported descriptions of

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premonitory patterns of seismicity. These patterns are of three basic types—quiescence, activation and migration of epicentres. Although the results from some of these studies support the hope that premonitory patterns of seismicity exist, nevertheless, with a few exceptions, their definition is not specific enough to estimate the reliability of the pattern. To obtain such estimates one should be able to detect the pattern by a formal algorithm and to count how often it is successful as a precursor. Without such estimates of reliability, any premonitory pattern would be uncertain.

We present here an attempt to derive a formal definition of some premonitory seismicity patterns from the analysis of an earthquake catalogue, which yields a reasonable success-to-failure ratio in retrospective long-term earthquake prediction. The definition also provides constraints on the theory of earthquake occurrence.

One reason why the search for premonitory patterns of seismicity has not been successful is that aftershocks are usually disregarded as being highly predictable phenomena. The results we describe below show that, while aftershocks are indeed highly predictable, some specific traits of aftershock sequences are highly informative. These traits may represent important, descriptive aspects of the distribution and redistribution of stresses and strengths within the Earth.

Definitions

Let t be the origin time of an earthquake, λ, ϕ the longitude and latitude of its epicentre (west and south are negative), h its focal depth, and M its magnitude. The subscript j will indicate the sequence number of an earthquake in the catalogue, $t_j < t_{j-1}$.

We seek precursors of earthquakes with magnitudes $M \geqslant M_0$. These are called strong earthquakes. Our patterns will be constructed from earthquakes in the magnitude range $M_2 \equiv M_0 - \mu_2 \leqslant M \leqslant M_0 - \mu_1 \equiv M_1$ and in a time interval of duration s; the values of μ_1, μ_2 and s may be different for each of the three patterns that we will discuss.

Aftershocks are defined as follows. Consider two earthquakes with sequence numbers i and j, $j > i$. The second earthquake is an aftershock of the first if the following conditions are satisfied: the distance between their epicentres is less than $R(M_i)$; $t_j - t_i \leqslant T(M_i)$; $h_j - h_i \leqslant H(M_i)$; $M_j \leqslant M_i$. Here $T(M)$, $R(M)$ and $H(M)$ are empirical functions. If we apply this definition to an earthquake catalogue, starting with the first earthquake, we can separate the catalogue into main shocks and their aftershocks. The first earthquake of the catalogue is identified as a main shock. Each succeeding main shock can be identified after the deletion of aftershocks of the preceding main shocks. Formulas of this type have been developed[1-3] for both the worldwide and the southern California[4] catalogues for use in other aspects of the study of aftershocks and sequences or chains of interdependent earthquakes.

Pattern B (burst of aftershocks). Let $b_i(e)$ be the number of aftershocks following the ith main shock within time interval e. We consider only those main shocks with magnitudes between M_2 and M_1 and only aftershocks with magnitudes at or above some threshold $m \equiv M_0 - \mu_3$. We invoke the thresholds M_2 and m for practical reasons as all catalogues are incomplete and inhomogeneous for small magnitudes. However, these thresholds may be of some value in explaining physical relationships, as the details of premonitory patterns may be different in different magnitude ranges.

Pattern B consists of a main shock and its aftershocks for which $b_i \geqslant C$. A more flexible definition of a threshold for b_i can be given[5], but it is not required for the present purposes.

An alarm is declared for a period of τ years after pattern B is diagnosed. A strong earthquake is expected during this period, having been predicted by pattern B. The alarm is terminated either at the time a strong earthquake takes place or at the end of this period, whichever comes first.

Pattern S ('swarms') was introduced elsewhere and its parameters were adjusted to the Italian earthquake catalogue[6]. We count the number of earthquakes in a time-window from $(t-s)$ to t, in the same magnitude range as above and in a restricted depth range. Let $N(t)$ be the total number of such earthquakes in the region. A reduced number of earthquakes $n(t)$ is obtained from $N(t)$ by the elimination of aftershocks of strong earthquakes. We then consider the map of the epicentres that are counted to determine $n(t)$. From this map we determine a third function $r(t)$ which is the maximum number of epicentres that can be surrounded by a small rectangular cell of dimensions $\Delta\phi$ in latitude and $\Delta\lambda$ in longitude.

Pattern S consists of a group of earthquakes such that $n(t) > \max\{C_1, C_2\bar{N}(t)\}$ and $r(t) > C_3 n(t)$. $\bar{N}(t)$ is the average of $N(t)$ over the interval t_0 to t or from $(t-ks)$ to t. The threshold M_1 plays no part in the identification of this pattern.

Pattern Σ has been introduced elsewhere[7] and its parameters were adjusted to some of the strongest earthquakes of the world. It is represented by a peak of the function $\Sigma(t) = \sum_j G(M_j)$ where the summation takes place over the same band of time and magnitude intervals as above. Aftershocks of strong earthquakes are eliminated from the summation. $G(M)$ is specified[7]

Table 1 Score of patterns B

Regions	1 Southern California	2 N. California–Nevada (excluding Mendocino area)	3 Southern Japan	4 Northern Japan	5 New Zealand	Totals
Catalogue ref.	4, 11, 12	9	10, 14	10, 14	13	
Span of catalogue (yr)	1932–78	1945–76.5	1926–76.5	1940–76.5	1945–77	201.5
M_0	6.5	6.2	8	7.6	6.6	
C	11–13	6–12	4–10	4–6	9–14	
Strong earthquakes						
total	6	4	2	4	7	23
'predicted'	5	3	2	4	4	18
Relative time of alarms	0.46	0.27	0.18	0.36	0.27	0.31
N_m	36	39	9	45	65	194
N_A/N_B	9/14	3/6	2/4	7/13	7/8	28/45
T_τ/T	0.37	0.28	0.10	0.27	0.53	0.30
P	96%	79%	95%	96%	95%	

N_m, total number of main shocks; N_B, number of main shocks which generate pattern B $b_i \geqslant C$, for upper value of C; N_A, number of occurrences of pattern B which are followed by strong earthquakes within τ yr; T_τ, sum of times τ before each strong earthquake, counting overlapping parts only once; T, span of the catalogue (yr); C, threshold for identification of pattern B. A variation of C within the limits indicated in the table will introduce either zero or one additional error; P, confidence level for upper value of C.

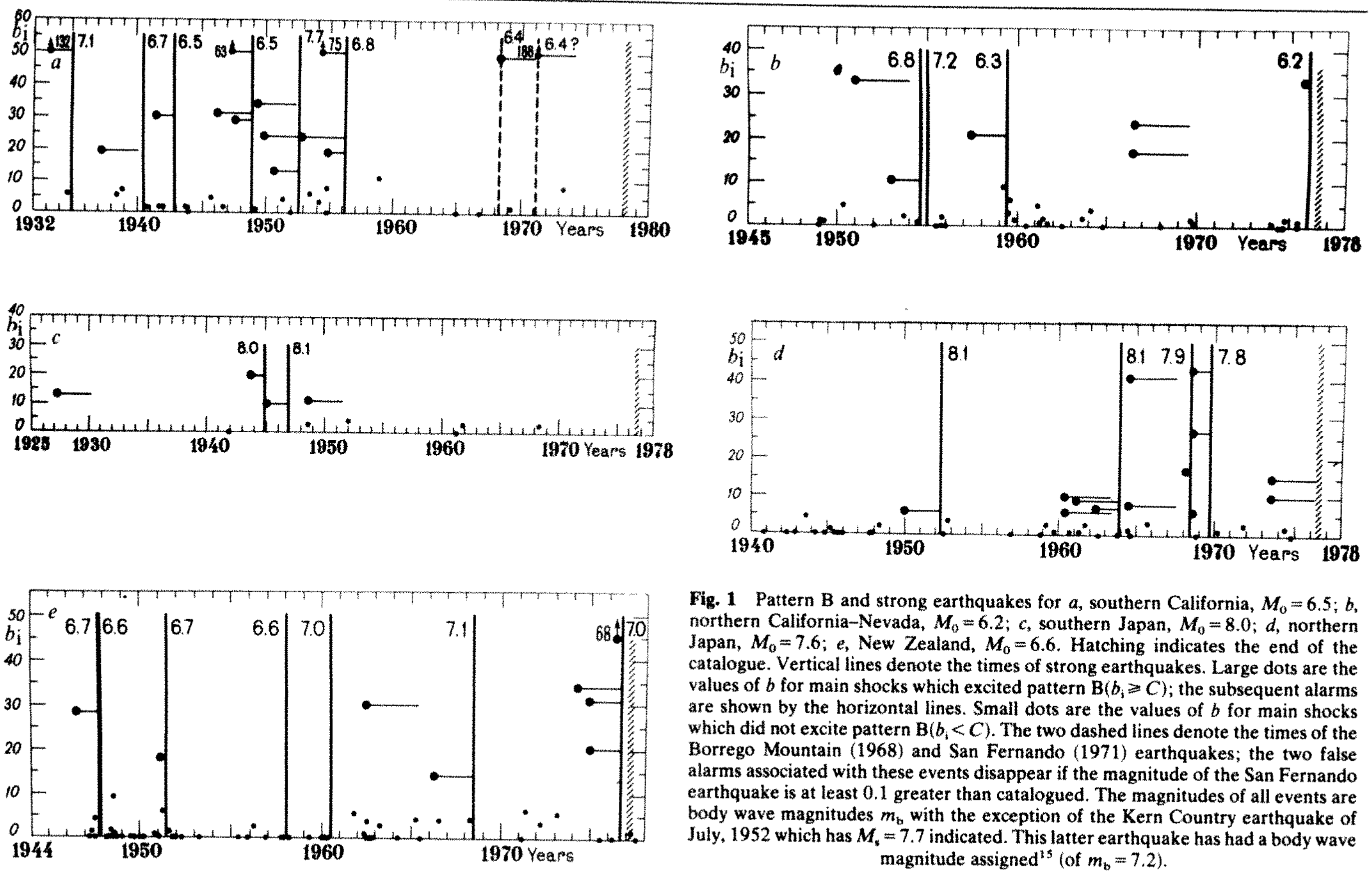

Fig. 1 Pattern B and strong earthquakes for *a*, southern California, $M_0 = 6.5$; *b*, northern California–Nevada, $M_0 = 6.2$; *c*, southern Japan, $M_0 = 8.0$; *d*, northern Japan, $M_0 = 7.6$; *e*, New Zealand, $M_0 = 6.6$. Hatching indicates the end of the catalogue. Vertical lines denote the times of strong earthquakes. Large dots are the values of *b* for main shocks which excited pattern B($b_i \geq C$); the subsequent alarms are shown by the horizontal lines. Small dots are the values of *b* for main shocks which did not excite pattern B($b_i < C$). The two dashed lines denote the times of the Borrego Mountain (1968) and San Fernando (1971) earthquakes; the two false alarms associated with these events disappear if the magnitude of the San Fernando earthquake is at least 0.1 greater than catalogued. The magnitudes of all events are body wave magnitudes m_b with the exception of the Kern Country earthquake of July, 1952 which has $M_s = 7.7$ indicated. This latter earthquake has had a body wave magnitude assigned[15] (of $m_b = 7.2$).

to be $G(M) = 10^{d(M-f)}$ where *d* and *f* are numerical parameters. Pattern Σ consists of earthquakes satisfying the condition $\Sigma(t) \geq \bar{\Sigma}$. The values $\bar{\Sigma} = cG(M_0)$, $c = 0.5$, $d = 0.91$ have been assumed elsewhere[7].

Alarms after patterns S and Σ are declared in the same manner as indicated after pattern B.

Data processing

We have tested pattern B in a retrospective 'prediction' of strong earthquakes in the five regions indicated in Table 1. Arbitrary decisions have had to be made regarding the definition of the geographical boundaries of these regions as well as the values of all parameters. No *a priori* theoretical limits can be imposed on these decisions and we have to obtain them by data fitting. This creates a danger of self-deception as the number of these decisions is rather large. To decrease this danger we have tried to make these decisions simple, uniform and, if possible, *a priori*. Also we have accepted, when possible, the arbitrary choices of parameters or thresholds made in previous studies, and we have tested the stability of our results with regard to variations in each of these choices. We have considered only those earthquakes with $h \leq 100$ km.

We have assigned the following values: $\tau = 3$ yr; $e = 2$ days; $\mu_1 = 0.1$; $\mu_2 = 1$; $\mu_3 = 3.5$. These values were obtained by data fitting for southern California[5]. To define aftershocks we took $R = 50$ km; $T(M) = 0.5$ years for $5.0 \leq M \leq 5.4$, $T(M) = 1$ yr for $5.5 \leq M \leq 6.4$ and $T(M) = 2$ yr for $M \geq 6.5$. The values of $R(M)$, $T(M)$ given elsewhere[3] for the southern California catalogue give better results, but these fine gradations are hardly warranted in view of the accuracy of some of the other catalogues used in this study. The values of $R(M)$ and $T(M)$ assumed here have the advantage that they can be used for a fast diagnosis of pattern B even before the epicentres of aftershocks are determined precisely.

The values of M_0 and *C* listed in Table 1, have been chosen separately for each region due to lack of experience and a satisfactory theory. The fact that these two parameters have been chosen *a posteriori* contributes the single largest difficulty in the evaluation of the significance of our results.

We have chosen the other parameters to be the same for all regions, even if they are unreasonably simple. This suffices for our present purpose which is to test the existence and stability of pattern B. For actual predictions, the parameters could probably be better adjusted to match the specific geophysical characteristics of the region as well as its own earthquake catalogue. More detailed studies of pattern B in some specific regions will be published elsewhere[8].

The boundaries of the southern California as well as the northern California–Nevada regions are defined elsewhere[4,9]. We straightened these boundaries slightly from the original definitions, and have deleted the area around Cape Mendocino from the northern California study as it has a distinctly isolated cluster of epicentres. The boundaries of the other regions are based on large-scale neotectonics and on the densities of epicentres.

Results

The results are summarised in Table 1. Examples of the details are documented in Table 2 and displayed in Fig. 1. The total span of all five catalogues is 202 yr. Twenty-three strong earthquakes occurred in the five regions during these time intervals. Eighteen of these earthquakes were retrospectively predicted, that is, they took place during the intervals of alarms sounded after each instance of pattern B. The total duration of these alarms is 60 yr or 30% of the total span of the catalogues. If we exclude the results for southern California, which were highly involved in the data fitting, that is, in the adjustment of parameters, we have 13 out of 17 strong earthquakes predicted for the remaining four regions; the total duration of alarms is 39 out of 155 yr or 25% of the total time.

Although these numerical values illustrate the practical efficiency of pattern B as a tool in prediction, they cannot be used in statistical tests of the existence of pattern B as, by the definition above, an alarm is terminated by the occurrence of a strong earthquake. To perform this statistical test (of the existence of pattern B), the values of the three parameters, N_A, N_B, and T_τ/T were tabulated and listed in Table 1.

The probability that a random binomial process could do as well or better than pattern B in anticipating strong earthquakes is given by the quantity $(1-P)$. This is the probability that N_A events or more will occur out of N_B tries in the fraction T_τ/T of the total catalogue time T according to a random binomial process. The values of P strongly support the hypothesis that B is a premonitory pattern, although these values of probability should only be used in a qualitative sense rather than literally. The fact that some thresholds were chosen *a posteriori* leads to an overestimation of P. On the other hand, the fact that some thresholds are chosen to be the same for all regions, leads to an underestimation of P. For example, the earthquake in southern Japan of 28 June 1948 could be deleted as a cause of a false alarm (Table 2) either by (1) changing e from 2 to 7 days, and/or (2) changing m to 5.0 from 4.5. Both of these changes are not unreasonable: in the first instance because $M_0 = 8$ is especially large and in the second because the catalogue[10] is more reliable for magnitudes greater than 5 than it is for magnitudes between 4.5 and 5. The last two false alarms for the southern California catalogue (Fig. 1) disappear if we assume that the magnitude of the San Fernando earthquake of 9 February 1971 is 6.5 instead of 6.4; this question is discussed elsewhere[5]. Similarly, the values of P could be increased by adjusting some of the parameters to take into account specific traits of the observed seismicity for each region separately. However, these adjustments would be non-unique; for the purpose of testing the existence of pattern B as a precursor, it is better to keep the criteria for all regions as uniform as possible, and that we make them, insofar as possible, *a priori*, and hence avoid tampering with the results by intuitive 'improvements' after inspection of the tabulations.

In the case of the northern California–Nevada catalogue we took $m = 3$, instead of $m = 2.7$ which would have been obtained from a literal application of our uniform definition $\mu_3 = 3.5$; this catalogue is especially inhomogeneous for $m < 3$. The errors in prediction with $m = 2.7$ and $m = 3$ are the same.

Table 2 lists the strong shocks as well as the main shocks that trigger pattern B; the strong shocks are underlined. Thus it is possible to see whether or not a strong shock was preceded by pattern B within 3 yr. Table 2 shows that our results remain essentially unaltered when the value of R is changed to 100 km and/or e to 7 d. Similar results have been obtained for the other catalogues.

We have indicated in Table 2 those events that would have been identified as aftershocks of the event immediately preceding had we used $R = 100$ km; deletion of these events from our listing would have improved our success rate as these invariably result in a reduction in N_B without a corresponding decrease in N_A. There are two examples of entries of this type in the southern California catalogue and none in the northern California and New Zealand catalogues.

A more detailed analysis of the stability of pattern B as well as the choice of the optimum parameters for the diagnosis of pattern B requires a separate study for each region. We have tried here to emphasise that pattern B can score quite satisfactory success-to-failure ratios, even with parameters that are uniform for all regions, despite the fact that each region has widely different tectonics, seismicity and quality of earthquake catalogue from the others.

Table 2 Pattern B and strong earthquakes

Month-day-year	epicentre ϕ	λ	M	b_i $R = 50(100)$ km		δT (yr)	n_m
				$e = 2$ d	$e = 7$ d		
Southern Japan Polygon: (30; 130), (33; 139), (39; 136), (33; 127.5)							
3- 7-27	35.6	135.1	7.5	13	18	7.8	
9-10-43	35.5	134.2	7.4	20	25	1.24	
12- 7-44	33.7	136.2	8.0	3	3		3
1-13-45	34.7	137.0	7.1	10	17	1.94	
12-21-46	33.0	135.6	8.1	2	3		1
6-28-48	36.1	136.2	7.3	11	11	⩾30	
Northern Japan Polygon: (33; 139), (37; 145), (40; 146), (45; 154), (49; 150), (45; 140), (39; 136)							
12-26-49	36.7	139.7	6.7	6	7	2.19	
3- 4-52	42.2	143.9	8.1	6(21)	9(28)		14
3-21-60	39.8	143.5	7.5	10(13)	19(30)	3.56	
3-23-60	39.3	143.8	6.7	6	6	3.56	*
1-16-61	36.0	142.3	6.8	9(13)	9(13)	2.74	
4-12-62	38.0	142.8	6.8	7(8)	10(12)	1.50	
10-13-63	43.8	150.0	8.1	4(8)	9(16)		15
5- 7-64	40.3	139.0	6.9	8	10	4.03	
6-16-64	38.4	139.2	7.5	41(42)	48(52)	3.92	
1-29-68	43.2	147.0	6.9	17(21)	23(33)	0.30	
5-16-68	40.7	143.6	7.9	12(45)	17(65)		6
5-16-68	41.4	142.8	7.5	27	50	1.24	*
5-17-68	39.8	143.5	6.7	6	7	1.24	
6-12-68	39.4	143.1	7.2	43(45)	51(55)	1.17	
8-12-69	42.7	147.6	7.8	7(37)	18(66)		4
6-17-73	43.0	146.0	7.4	15(30)	18(45)	>4	
6-24-73	43.0	146.8	7.1	10(30)	16(45)	>4	*

Values of b_i for $R = 100$ km are indicated only if they are not the same as for $R = 50$ km; δT, time from the given pattern B until the next strong earthquake; n_m, the number of all main shocks between the given and preceding strong earthquakes, or since the beginning of the catalogue in the case of the first strong earthquake; * denotes a main shock, that would have been identified as an aftershock with $R = 100$ km.

A speculation on generalisation

The same catalogues were processed to detect the presence of patterns S and Σ (Fig. 2). The value of M_0 is assumed to be the same as for pattern B. As in the papers where these patterns were introduced[6,7], the parameters were taken to be as follows: $C_1 = 0$, $C_2 = 1$, $C_3 = 0.5$, $\mu_2 = 2$, $\mu_1 = 0.5$ for Σ (absent for S), $d = 0.91$, $f = 4.5$, $s = 1$ yr for S. For Σ we varied $\bar{\Sigma}$ and s for each of the regions (Fig. 2). The absence of a pattern signifies that it could not be detected for the given region. We added data for Alaska in view of the striking success of pattern Σ.

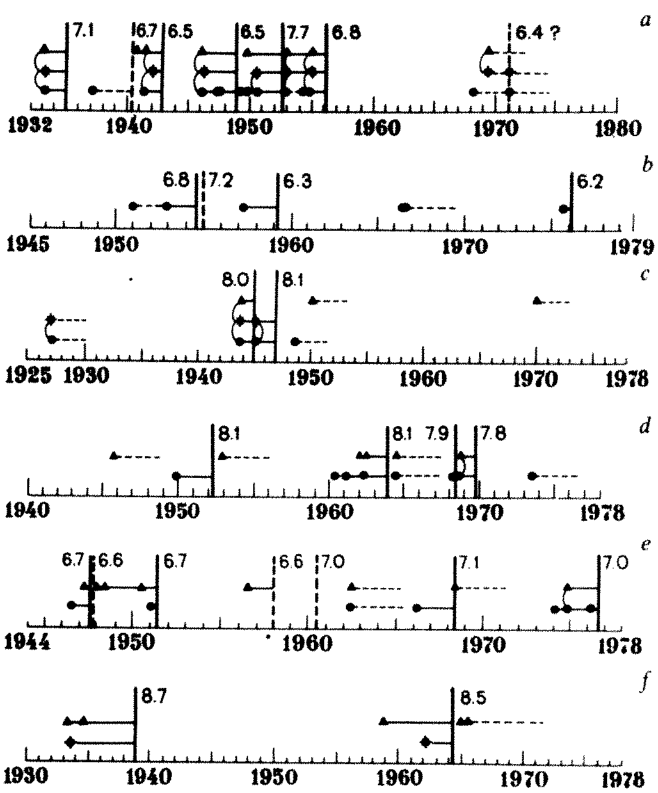

Fig. 2 Occurrence of patterns B (●), S (◆) and Σ (▲).

	$\bar{\Sigma}$	s (yr)	$\Delta\phi$	$\Delta\lambda$
a, Southern California	60	1	0.4°	0.6°
b, Northern California–Nevada				
c, Southern Japan	900	3	0.4	0.6
d, Northern Japan	700	1	1.0	1.4
e, New Zealand	95	0.5	0.4	0.6
f, Alaska	770	2	0.5	1.0

Solid horizontal line is period of alarm; dashed is false alarm. Solid vertical line is time of strong earthquake; dashed is strong earthquake not predicted by pattern B. Brackets denote different patterns generated by the same main shock and the appropriate parts of its aftershock series.

In several cases the same main shock and its aftershocks will give rise to several different patterns from among Σ, S, B. This result is not unreasonable as by definition, a main shock and its aftershocks will generate:

(1) pattern B if the number of aftershocks during the first e days is large enough;

(2) pattern S if the number of aftershocks during the time interval s, or during any part of it, is large enough; we usually take $s = 1$ yr;

(3) pattern Σ if a sufficiently large number of events have magnitudes close to the upper limit M_1.

In most cases in southern California the same main event plus its aftershocks generate two or even all three patterns. Moreover, the coincidence of a combination of two or three of the patterns eliminates false alarms without adding any failures-to-predict[5].

Patterns Σ and S also appear in conjunction before extremely strong earthquakes in the Tibet–Himalaya region[8]; in this region pattern B cannot be diagnosed, as the catalogues that are available do not include aftershocks.

We find that prediction by pattern B is successful when two conditions are satisfied: not only is a large number of aftershocks b_i required but also the main shock producing it must have a large enough magnitude; if the magnitude of a main shock is too low, but is still followed by an exceedingly large b_i, a false alarm will be generated. We have therefore introduced the threshold M_2. On the other hand we find both the numbers of false alarms and failures-to-predict increase significantly if we ignore the number of aftershocks produced by a main shock and merely consider the occurrence of a main shock itself as a possible precursor: for example, these numbers increased when we considered as a premonitor any earthquake with magnitude up to M_0, that is, by removing the gap between M_2 and M_0, by increasing M_1 and ignoring the threshold C.

We can imagine that a hypothetical generalised premonitory pattern exists which is an abnormal clustering of earthquakes in a space–time–energy domain. We call this hypothetical pattern a 'burst of seismicity'. Our three particular patterns may represent different projections of this generalised pattern. The definitions of the three are neither unique nor optimised; the use of discrete magnitude thresholds, especially in regard to large uncertainties in magnitudes of earthquakes in the catalogues, is an inadequate and troublesome aspect of the work so far. Our results indicate, however, that these patterns exist and deserve attention.

We have refrained from providing additional refinements, such as geological regionalisations or to assign probabilities to the forecasts as functions of time to the forthcoming event and its magnitude. The results of the present exploration indicate that such refinements are necessary and we hope that they would improve our success ratios, which are surprisingly high despite the simplicity of the criteria we have used.

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Regulation of acetylcholinesterase appearance at neuromuscular junctions *in vitro*

Lee L. Rubin*, Stephen M. Schuetze†, Cheryl L. Weill‡ & Gerald D. Fischbach

Department of Pharmacology, Harvard Medical School, 250 Longwood Avenue, Boston, Massachusetts 02115

The appearance of acetylcholinesterase (AChE) at newly formed nerve–muscle synapses depends on synaptic transmission. Synapses form when cultures are grown in the presence of acetylcholine receptor antagonists, but AChE does not accumulate at these synapses. The important component of transmission seems to be muscle activity. Treatment with dibutyryl cyclic GMP mimics muscle activity, directly inducing synaptic AChE appearance.

ACETYLCHOLINE receptors (AChRs) and acetylcholinesterase (AChE) are concentrated at adult motor endplates. The AChR is an integral membrane protein (see ref. 1 for review) restricted to the upper one-third of the postjunctional folds, whereas AChE is a peripheral protein associated with the basal lamina[2] throughout the primary and secondary synaptic clefts[3]. Even though AChRs and AChE are ultimately located at different sites, both molecules begin to accumulate within 24 h of nerve–muscle synapse formation *in vitro*[4–6] and are present in approximately equal numbers at mature junctions [3,7,8]. Therefore, one might suppose that the early AChR and AChE appearance at endplates is regulated in a coordinated way. Certain observations suggest, however, that synaptic AChRs and AChE are influenced quite differently by chronic blockade of synaptic transmission. As AChRs cluster at nerve–muscle contacts when spinal cord–muscle co-cultures are grown in the presence of α-bungarotoxin (α-BTX)[9], this process is independent of synaptic transmission. On the other hand, discrete foci of AChE fail to appear when young chick embryos are injected repeatedly with α-neurotoxin or curare[10,11]. These results have been taken as evidence that receptor antagonists block synapse formation. It is possible, however, that nerve–muscle synapses form in the presence of receptor antagonists (compare refs 12, 13) but that AChE does not accumulate in the absence of synaptic transmission. Data presented here indicate that this, in fact, is the case and, further, that activity of innervated muscle fibres is a crucial parameter regulating the early appearance of AChE at sites of transmitter release. The accumulation of AChRs and of AChE during neuromuscular junction formation is apparently not regulated in exactly the same way. A preliminary account of some of these experients has been given elsewhere[14].

As AChE accumulates rapidly at newly formed chick nerve–muscle synapses *in vitro*[6], we used this culture system to examine in more detail the influence of synaptic transmission and muscle activity on AChE. Nerve processes emerge from spinal cord explants 10 h after the fragments attach to the culture surface, and synapses form soon after nerve–muscle contact. We have shown previously that AChE can be detected histochemically at some physiologically identified sites of transmitter release within 24 h of the onset of synaptic transmission and that after 4 d of co-culture, AChE reaction product is present at the majority of functional contacts[6]. Synaptic AChE activity was also detected by measuring the time course of synaptic currents recorded with an extracellular electrode. At adult endplates, AChE serves to terminate transmitter action, and this effect is reflected in the rate of synaptic current decay[15–17]. Based on the mean time constant of synaptic current decay ($\bar{\tau}_{syn}$) recorded at 30 °C, synapses formed in culture were arbitrarily divided into three categories: fast ($\bar{\tau}_{syn} \leq 1.8$ ms), intermediate ($1.8 \text{ ms} < \bar{\tau}_{syn} < 2.6$ ms) and slow ($\bar{\tau}_{syn} \geq 2.6$ ms). Whereas 74% of the fast synapses stain for AChE, none of the slow ones stain.

Curare blocks appearance of synaptic AChE

Chick spinal cord explant–muscle co-cultures were prepared as previously described[5,6]. Thin slices cut from the brachial segments of 14-d embryonic cords were added to established muscle cultures. Curare at a final concentration of 50 µM was added to some cultures for 3–4 d beginning at the time of explant addition. Innervated myotubes in control medium twitched vigorously and were continuously active. At 50 µM, curare blocked all synaptic activity and only a few isolated contractions were observed. This high concentration of curare did not block

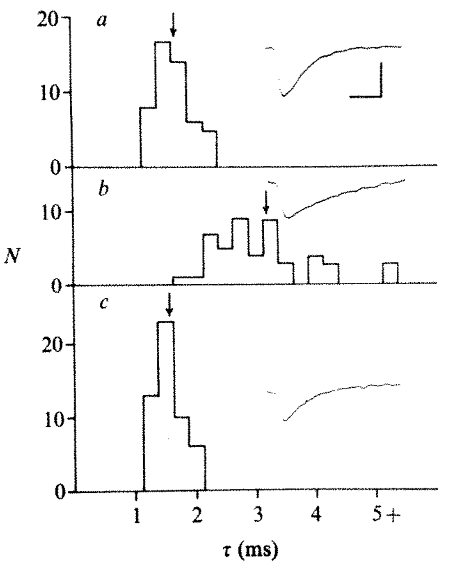

Fig. 1 Histograms of synaptic current decay time constants (τ_{syn}) recorded at 30 °C. Representative synaptic currents recorded with an extracellular microelectrode are shown as insets. Time constants were determined from exponential curves fitted to each synaptic current decay phase with an on-line computer. Arrows above each histogram indicate arithmetic means ($\bar{\tau}_{syn}$). At the control synapse (*a*), $\bar{\tau}_{syn}$ is <1.8 ms, whereas at the synapse formed and maintained in the presence of curare (50 µM for 4 d; *b*), $\bar{\tau}_{syn}$ is >2.6 ms. Both $\bar{\tau}_{syn}$ and the variance of τ_{syn} are increased at the curare-treated synapse, reflecting the lack of synaptic AChE activity[6,17]. After data in *b* were obtained, the culture was placed in control medium without curare and returned to the incubator. Two days later, *c* was obtained by recording at another synapse in the same culture. $\bar{\tau}_{syn}$ and the variance of τ_{syn} were then comparable to control. Calibration bars, 2 ms and 200 µV.

Present addresses: * Department of Neurobiology, Stanford University Medical School, Stanford, California 94305; † Department of Anatomy, Harvard Medical School, 25 Shattuck Street, Boston, Massachusetts 02115; ‡ Department of Neurology, Louisiana State University Medical School, 1542 Tulane Avenue, New Orleans, Louisiana 70112.

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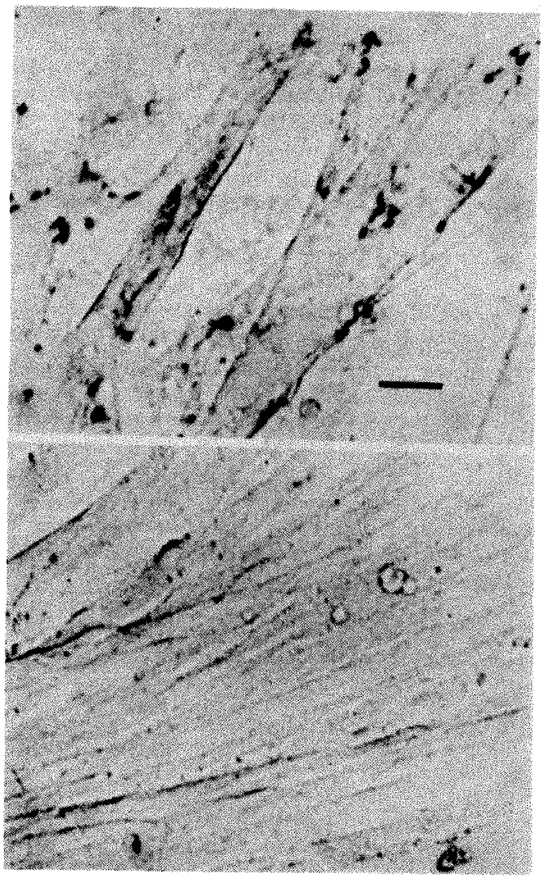

Fig. 2 Bright field view of control (upper) and 50 μM curare-treated (lower) myotubes stained for AChE according to the method of Karnovsky and Roots[32] 4 d after addition of spinal cord explants. Both fields were near (within 300 μm of) the explants. Many patches of reaction product near fine nerve processes (not visible under bright field illumination) are evident on control myotubes. Few, if any, patches are present on curare-treated myotubes. Bar, 100 μm.

the formation of synapses. Spontaneous synaptic activity was detected in most myotubes within 1–2 min of curare washout. In addition, autoradiographs of cultures treated with ^{125}I α-BTX immediately after curare washout showed that AChRs had clustered at sites of transmitter release.

Although synapses formed in the presence of curare, extracellularly recorded synaptic currents decayed relatively slowly (Fig. 1), indicating that AChE did not accumulate at sites of transmitter release. Judging from histograms of τ_{syn} constructed at 97 control and 89 curare-grown synapses (immediately after curare washout), the percentage of fast synapses decreased fivefold, whereas the percentage of intermediate synapses increased twofold and that of slow synapses threefold (Table 1). In the intermediate group, there was a shift towards higher values ($\bar{\tau}_{syn} > 2.2$ ms). The effect of curare was reversible; 1–2 d after cultures were returned to control medium, synaptic currents at most synapses decayed rapidly (Fig. 1).

Prolonged synaptic currents following curare washout were not due to an increase in the mean ACh channel open time. Estimates based on spectra of ACh-induced current fluctuations at control and curare-grown synapses were not significantly different (Table 1).

Histochemical experiments confirmed electrophysiological findings. The number of patches of AChE reaction product on myotubes near spinal cord explants in curare-grown cultures was reduced more than 10-fold compared with control (Fig. 2, Table 1). It cannot be assumed that all AChE patches are located at synapses, so sites of transmitter release were identified by extracellular recording, and then the same sites were relocated after fixation and histochemistry. Reaction product was evident at 60 of 94 control synapses, but at only 1 of 49 synapses that had formed in the presence of curare. The reversibility of the curare inhibition illustrated above was also confirmed histochemically. Many AChE patches were evident 1–2 d after return to control medium.

Curare at 50 μM did not affect the outgrowth of neurites, but in some cultures the nerve processes were more heavily stained for AChE than in control cultures. Chronic treatment with 2×10^{-8} M α-BTX also eliminated patches of AChE at synapses on innervated myotubes, but did not increase the extent of neuronal staining.

Curare blocks the appearance of 19.5S AChE

Several forms of AChE can be detected by sucrose density gradient centrifugation of extracts prepared from adult muscle (see ref. 18 for review). A high-molecular weight form sedimenting at 16S in rat[19] and 19.5S in chick[20] extracts is present in innervated but not in denervated muscle. With our method of extraction (see Fig. 3 legend), we have never detected 19.5S AChE in chick muscle cultures without added neurones or in spinal cord cultures without target myotubes, nor have we reliably detected 19.5S AChE in spinal cord explant–muscle co-cultures; the number of synapses formed may be too small. However, as shown in Fig. 3*a*, a 19.5S peak was evident in muscle cultures seeded with a large number of dissociated spinal cord cells (see ref. 21); two other peaks of AChE sedimented at 7S and 11S. In different experiments, the 19.5S peak comprised 5–10% of the total activity. In five experiments, chronic exposure to curare decreased activity in the 19.5S peak at least fourfold (Fig. 3*b*) without decreasing the total AChE-specific activity (control, 0.314±0.31 μM per min per mg; curare, 0.395±0.5 μM per min per mg). The heavy form of AChE is restricted to the endplate region of innervated rat muscle[19], but it is found in endplate-free regions of innervated chick muscle[20]. Therefore, although a useful marker for the 'innervated state', it is not clear if the 19.5S form accounts for the AChE activity at synapses detected histochemically and electrophysiologically in our cultures.

The appearance of AChE at synapses depends on muscle activity

The effect of curare and α-BTX implies that something in the sequence of events that follow ACh release—ACh binding, ACh-induced increase in membrane conductance, muscle excitation and muscle contraction—is required for the accumulation of synaptic AChE. ACh binding is probably not sufficient; synaptic AChE was drastically reduced by chronic exposure to 10 μM proadifen-HCl (SKF 525A). At this concentration, this local anaesthetic blocks ACh channels, but does not inhibit ACh binding[22]. Subthreshold synaptic conductance changes are probably not sufficient either; the appearance of AChE was substantially suppressed by tetrodotoxin, which blocks action potentials and, thus, impulse-mediated endplate potentials

Table 1 Comparison of control and curare-treated cultures

	$\bar{\tau}_{syn}$ (ms)			AChE patches	τ_0 (ms)
	1.0–1.8	1.8–2.6	>2.6	(mean ±s.e.)	(mean ±s.e.)
Control (97*)	59.8%	29.9%	10.3%	25.0±2.0 (41†)	1.34±0.08 (4‡)
Curare (89*)	12.4%	56.2%	31.5%	1.9±0.3 (58†)	1.49±0.16 (4‡)

Mean synaptic current decays ($\bar{\tau}_{syn}$) were determined at 30 °C as described in Fig. 1 legend. The number of patches of AChE reaction product (per spinal cord explant) was calculated by counting patches in the vicinity of neurites on undamaged myotubes. No more than 50 patches were counted on myotubes surrounding a single explant, so the control value is an underestimate. ACh channel open times (τ_0) were determined at fast synapses in control cultures and at slow synapses in curare-treated cultures using the technique described by Schuetze *et al.*[32].

* No. of synapses at which $\bar{\tau}_{syn}$ was determined.

† No. of explants.

‡ No. of synapses at which τ_0 was determined.

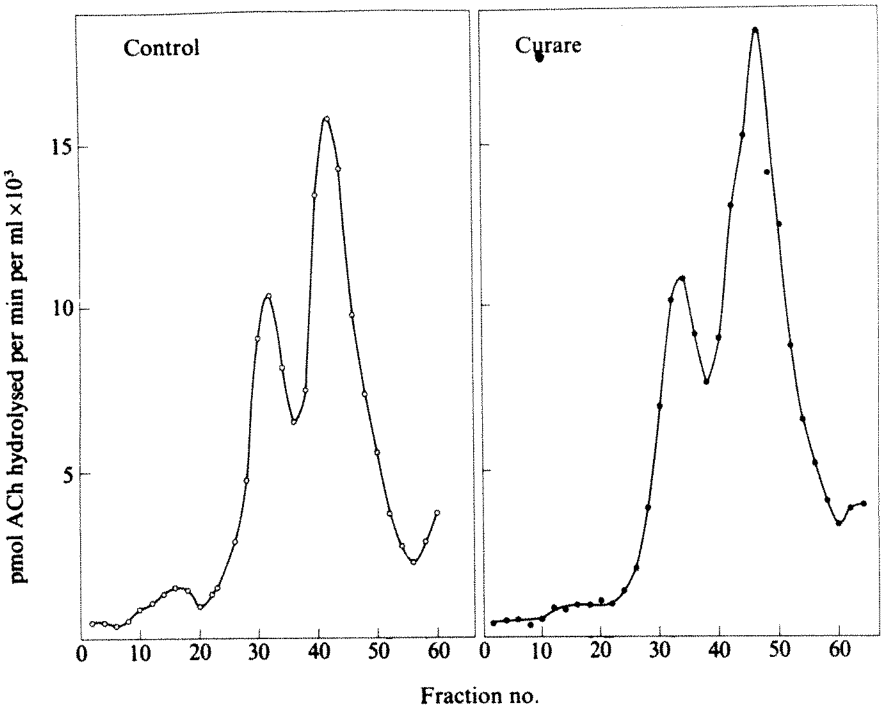

Fig. 3 Sucrose density gradient fractionation of different molecular forms of AChE. Chick muscles were plated at 3×10^5 cells per 60-mm culture dish. On day 3, 6-d embryonic chick spinal cords were dissociated and added ($2–3 \times 10^6$ cells per dish) to the muscle cultures in the presence of 10^{-5} M cytosine arabinoside with or without 50 μM curare. Five days later, three control and three curare-treated cultures were rinsed with Earle's balanced salt solution and the cells were collected by scraping with a Teflon policeman in phosphate-buffered saline containing 0.2 mM EDTA, *p*H 7.4. The cell suspension was centrifuged at 12,000*g* for 5 min and resuspended in 1 M NaCl, 0.5% Triton X-100, 0.05 M Tris-HCl, 0.2 mM EDTA and 1 mg ml^{-1} DNase (Sigma), *p*H 7.3. After 1 h at 0 °C, this suspension was centrifuged at 12,000*g* for 15 min and the supernatant assayed for protein[34] and AChE activity[35]. An aliquot (approximately 400–600 μg per 125 μl) was layered onto a 5–20% linear sucrose gradient and centrifuged in a Spinco SW41 rotor at 205,000*g* for 18 h at 4 °C. Sixty 0.25 ml fractions were collected and assayed for AChE by the radiometric method of Potter[36], as modified by Hall[19]. Three peaks of activity sedimenting at 7S, 11S and 19.5S were evident in the control gradient (left). Activity in the 19.5S peak was reduced at least fourfold by curare treatment (right). This figure shows the maximum 19.5S activity found in curare-treated cultures in five different experiments (platings). Gradient markers were alcohol dehydrogenase (4.8S), catalase (11.3S) and β-galactosidase (16S).

(e.p.p.s), but not impulse-independent miniature endplate potentials. Moreover, AChE did not accumulate in low concentrations (3 μM) of curare, which only reduced the mean e.p.p. by 50%.

Although 3 μM curare did not completely block e.p.p.s, most of the synaptic potentials were subthreshold; few innervated fibres were mechanically active. We therefore examined the role of muscle activity in the induction of synaptic AChE by electrically stimulating individual muscle fibres in the presence of curare. Spinal cord–muscle cultures were grown in 50 μM curare, and $\bar{\tau}_{syn}$ was again determined at previously identified sites of transmitter release. Chronic stimulation in the presence of curare significantly decreased τ_{syn} (Fig. 4). Eight synapses at which $\bar{\tau}_{syn}$ was greater than 2.21 ms were studied before and after stimulation (Table 2). Chronic stimulation in the presence of curare significantly decreased $\bar{\tau}_{syn}$ at seven of these synapses to values typical of fast, AChE-positive sites. One other synapse was fast ($\bar{\tau}_{syn} = 1.54$ ms) before stimulation, and its decay constant did not decrease further.

Influence of dibutyryl cyclic GMP on AChE

During these experiments, we found that low concentrations (25–100 μM) of dibutyryl cyclic GMP can reverse the curare inhibition of AChE accumulation. $\bar{\tau}_{syn}$ was determined at 114 synapses in cultures grown in 50 μM curare with dibutyryl cyclic GMP: 64% were fast and only 3.5% were slow. These percentages are comparable to those obtained with untreated control cultures (Table 1). The effect of dibutyryl cyclic GMP was also evident when AChE was assayed histochemically. Reaction product was present at 70% of identified synapses in cultures of curare plus dibutyryl cyclic GMP. Finally, dibutyryl cyclic GMP reversed the curare inhibition of 19.5S AChE assembly (Fig. 5) without increasing the total AChE activity. In six experiments, the ratio of total AChE (control/dibutyryl cyclic GMP treated) was 1.15±0.25 (mean±s.d.). Dibutyryl cyclic GMP did not 'induce' 19.5S AChE in muscle cells or in spinal cord neurones grown alone. Other agents, including GMP, GTP and dibutyryl cyclic AMP, did not reverse the curare inhibition of synaptic AChE accumulation or assembly of the 19.5S form.

Table 2 Effect of direct muscle stimulation on $\bar{\tau}_{syn}$

Synapse no.	Before	After	% Change
1	2.21±0.70	1.74±0.32	21.3
2	3.09±1.39	1.74±0.39	43.7
3	2.25±0.73	1.35±0.20	40.0
4	3.06±1.04	2.05±0.71	33.0
5	3.19±1.56	1.87±0.49	41.4
6	3.14±0.87	1.75±0.37	44.3
7	2.59±1.15	1.80±0.41	30.5
8	2.42±0.92	2.30±0.65	5.0
9	1.54±0.35	1.51±0.21	1.9

$\bar{\tau}_{syn}$ (±s.d.) was determined from the time constants of decay of at least 20 extracellular potentials recorded at the same synapses in curare-treated cultures before and after direct electrical stimulation of myotubes in the presence of 50 μM curare for 8–12 h at 2–3 Hz and 35–36 °C.

Conclusions

The early accumulation of AChE at newly formed nerve–muscle synapses *in vitro* depends on synaptic transmission. By our physiological and histochemical criteria, the activity of the enzyme is greatly reduced at synapses that form in the presence of curare. Although 19.5S AChE is not restricted to adult chick endplates, it is significant that this form of the enzyme was selectively decreased in curare-blocked cultures.

Of the several events that follow synaptic transmission, muscle activity (electrical and/or mechanical) seems to be the crucial factor, as AChE does accumulate at curare-blocked synapses when innervated myotubes are stimulated directly. Lømo and Slater[23] have also emphasised the role of muscle activity in AChE accumulation at ectopic synapses on adult rat muscle fibres. Weinberg and Hall[24] suggested muscle activity as an explanation for their observation that 16S AChE (and AChE histochemical reaction product) reappears at denervated rat endplates when the denervated muscles are reinnervated at another site. Koenig and Vigny[21] detected 16S AChE in control rat spinal cord–muscle co-cultures but not in inactive cultures grown in the presence of tetrodotoxin[25]. Inadequate muscle activity may explain why no evidence was obtained for AChE activity at nerve–muscle synapses when chick muscle cultures were seeded with a relatively small number of dissociated spinal cord neurones[26]. In this system, innervated myotubes twitch more frequently than uninnervated myotubes, but the overall activity is far less than that observed in innervated myotubes near intact spinal cord explants.

In contrast to the situation with AChE, the clustering of AChRs in the postsynaptic membrane does not depend on activity. Thus, the formation of AChR clusters in the postsynaptic membrane and the accumulation of AChE in the synaptic cleft are not necessariinked. This result does not rule out the possibility that AChE and AChRs are synthesised and transported to the cell surface in a coordinated manner. Muscle activity may simply ensure the anchorage of AChE to the basal lamina of the synaptic cleft.

Muscle activity is probably not the sole determinant of synaptic AChE. Nerve extracts slow the rate of AChE disappearance, measured biochemically, from adult muscle maintained in organ culture[27,28], and saline extracts of central nervous tissue increase total AChE of uninnervated embryonic myotubes in culture[29,30]. Although it is difficult to compare these biochemical estimates of total (intracellular as well as extracellular) AChE with our physiological and histochemical assays, which detect AChE exposed on the cell surface at synapses, our emphasis on the importance of muscle activity is not inconsistent with the notion that synaptic AChE is also regulated by soluble factors supplied by motor nerves. The fact that AChE and AChRs are restricted to sites of transmitter release argues for a local neural influence. Moreover, we have identified AChE-positive and AChE-negative synapses within 500 μm of one another on the same myotube (Fig. 7 in Rubin *et al.*[6]). If both contacts were formed at approximately the same time and if both were exposed to the same degree of muscle activity, some additional factor must be required. Muscle activity may be 'permissive', allowing neuronal factors to exert their effects.

Addition of dibutyryl cyclic GMP to cultures grown in curare mimics the effects of muscle activity on synaptic AChE and on assembly of the 19.5S form of the enzyme. Of interest in this regard is the finding that stimulation of adult skeletal muscle results in an increase in cyclic GMP levels[31]. Neither result demonstrates a necessary connection between activity and cyclic GMP in regulating the appearance of AChE. Perhaps a stronger argument for such a connection could be made if muscle activity and cyclic GMP treatment resulted in phosphorylation of the same muscle proteins.

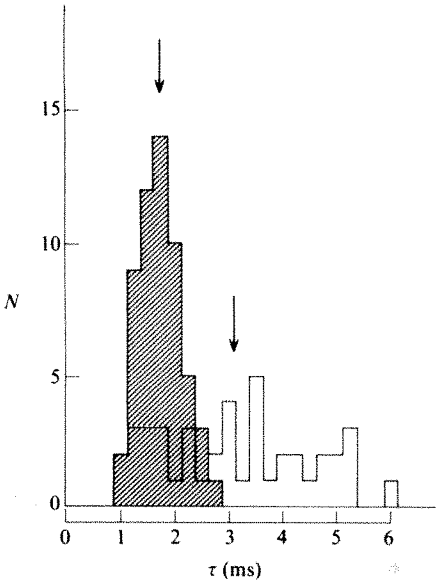

Fig. 4 Chronic muscle stimulation in the presence of curare decreases $\bar{\tau}_{syn}$. This culture was grown in 50 μM curare for 4 d, at which time synaptic current decays (recorded during a brief period of curare washout) at this synapse were prolonged (unshaded histogram). Immediately after the recordings were made, the culture was returned to medium with 50 μM curare, and the innervated myotube was electrically stimulated at 3 Hz for 11 h at 35–36 °C. Immediately after stimulation and curare washout, the same synapse was relocated and a clear decrease in $\bar{\tau}_{syn}$ was observed (shaded histogram). The variance of τ_{syn} was also decreased. All recordings were obtained at 30 °C. Arrows indicate $\bar{\tau}_{syn}$.

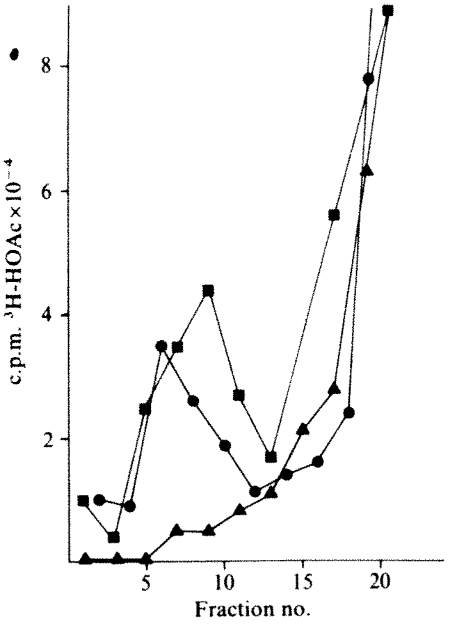

Fig. 5 19.5S AChE appears in spinal cord–muscle cell cultures grown in curare plus dibutyryl cyclic GMP. Cultures were grown without added drugs (●), with 50 μM curare (▲) or with 50 μM curare plus 100 μM dibutyryl cyclic GMP (■). Extracts were prepared and sucrose gradients run, as described in Fig. 3 legend. Only the high-molecular weight region of the gradient is shown. Assembly of 19.5S AChE was nearly completely blocked by curare, but this inhibition was reversed by adding dibutyryl cyclic GMP.

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Interactions between the carbohydrate chains of hyaluronate and chondroitin sulphate

E. A. Turley & S. Roth

Department of Biology, Johns Hopkins University, Baltimore, Maryland 21218

Hyaluronic acid-derivatised beads spontaneously agglutinate with chondroitin sulphate-derivatised beads although neither bead type shows any self-agglutination. The interaction between hyaluronate and chondroitin sulphate is specific for these two glycosaminoglycans and appears to occur between their carbohydrate chains.

ALMOST all mammalian cells contain glycosaminoglycans as a part of their coat and extracellular material[1]. All of the glycosaminoglycans so far studied, except hyaluronic acid, are sulphated and covalently linked to protein[1,2]. Hyaluronate, by contrast, is known to bind noncovalently to the protein portion of the proteoglycans[3]. The biological functions of these polymers are poorly understood. Glycosaminoglycans are apparently important structural molecules and have long been thought to function in the regulation of cation and H_2O binding as well as the control of macromolecular distribution by steric exclusion[4]. More recently, glycosaminoglycans have been implicated in cell adhesion[1,5,6], organogenesis[7], and differentiation[8]. In particular, the excellent correlation between cell migration *in vivo*[8–11] and *in vitro*[12] and the elevated levels of hyaluronate and chondroitin sulphate suggest that a cell–glycosaminoglycan interaction may be important for cell movement. Some of these biological effects may be due to the ability of the glycosaminoglycans to bind to cell surface components. Binding and chemical cross-linking studies[13] have indicated that the cell-surface glycoprotein fibronectin exists in close proximity to both hyaluronic acid and the sulphated glycosaminoglycans. This report demonstrates that chondroitin sulphate and hyaluronic acid are capable of a specific binding interaction with each other and this interaction occurs between the carbohydrate chains of the polymers. This observation raises the possibility that glycosaminoglycans located at the cell surface may function as binding sites and, therefore, be involved in cellular adhesive and recognition phenomena.

Derivatisation of the glycosaminoglycans

Solutions of chondroitin-4-sulphate (C-4-S), chondroitin-6-sulphate (C-6-S), hyaluronic acid (HA) dermatan sulphate, and heparin were dialysed and protein levels were shown to be <0.1%. The polymers were attached to Dowex AG 50 W-X-1 beads (50–100 mesh) by first converting the beads to the sulphonamide form and then incubating them with one of the

Table 1 Binding of chondroitin sulphate and hyaluronic acid to polysulphonamide beads

Concentration of glycosaminoglycan (μg per 100 mg beads, wet wt)	Glycosaminoglycan attached (μg per mg beads, dry wt)	Concentration of glycosaminoglycan (μg per 100 mg beads, wet wt)	Glycosaminoglycan attached (μg per mg beads, dry wt)
Chondroitin 6-sulphate		Hyaluronic acid	
15,000	100–130	15,000	25–30
1,500	10–12	1,500	12–15
150	1.0–1.4	150	5–7
15	0.1	15	1–5
1.5	<0.04		

Chondroitin-4-sulphate, chondroitin-6-sulphate, dermatan sulphate and heparin were purchased from Sigma Chemicals. Hyaluronic acid was a gift from Dr B. Toole. All the glycosaminoglycan solutions were dialysed for 24 h against distilled water, lyophylised and stored, desiccated, until use. To determine the degree of molecular weight homogeneity, the compounds were chromatographed on Sephadex G-200 in a buffer[14] containing 1% SDS (w/v), 10^{-4} M Na_2EDTA, 10^{-3} M dithiothreitol, 10^{-3} M sodium azide and 0.05 M phosphate buffer (*p*H 7.2). Fractions were assayed for carbohydrate with phenolsulphuric acid[15] and for protein by the Lowry method[16], by UV absorbance at 260 and 280 nm (ref. 17), 215 and 225 nm (ref. 18) and by the Bio-Rad protein assay[19]. Hyaluronic acid eluted in the Sephadex void volume whereas the other glycosaminoglycans were retarded and eluted as a single, symmetrical peak when assayed for carbohydrate. In assay conditions that could detect 10 μg protein, no protein was detectable in aliquots containing 10 mg of either chondroitin sulphate or hyaluronic acid. These carbohydrate preparations, therefore, contained less than 0.1% protein. To derivatise beads with the glycosaminoglycans, Dowex AG 50 W-X-1 (50–100 mesh) beads (Bio-Rad) were converted to the sulphonamide form by incubation in ammonium hydroxide (5 g beads per 10 ml NH_4 OH) at room temperature for 24 h. The beads were then washed exhaustively in distilled water and 0.1 g wet weight of the beads was added to 1 ml of an aqueous solution of either chondroitin-6-sulphate (0.0015–15 mg ml^{-1}) or hyaluronic acid (0.015–15 mg ml^{-1}). Each incubation also contained five times more (w/w) of the catalyst 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide (Sigma) than glycosaminoglycan. The glycosaminoglycan solution and beads were incubated for 48 h at 35–37 °C after which the beads were washed extensively in distilled water and, finally, in aseptic, phosphate-buffered saline (PBS) in which they were stored. Aliquots of the derivatised beads were analysed by subjecting the beads to hydrolysis in 90% formic acid at 100 °C for 6 h. The hydrolysates were evaporated to dryness and the resultant monosaccharides were assayed for uronic acid by the Dische assay[20] in the case of hyaluronic acid. To monitor the derivatisation of the chondroitin sulphates, tritiated chondroitin sulphates (1×10^{10} c.p.m. per mg) were prepared by galactose oxidase treatment followed by reduction with sodium borotritide as described previously[12]. The labelled compound was then used in the carbodiimide-catalysed derivatisation procedure. The hydrolysed monosaccharides were evaporated to dryness, redissolved in H_2O and counted in a scintillation counter. Values represent the ranges of two separate experiments.

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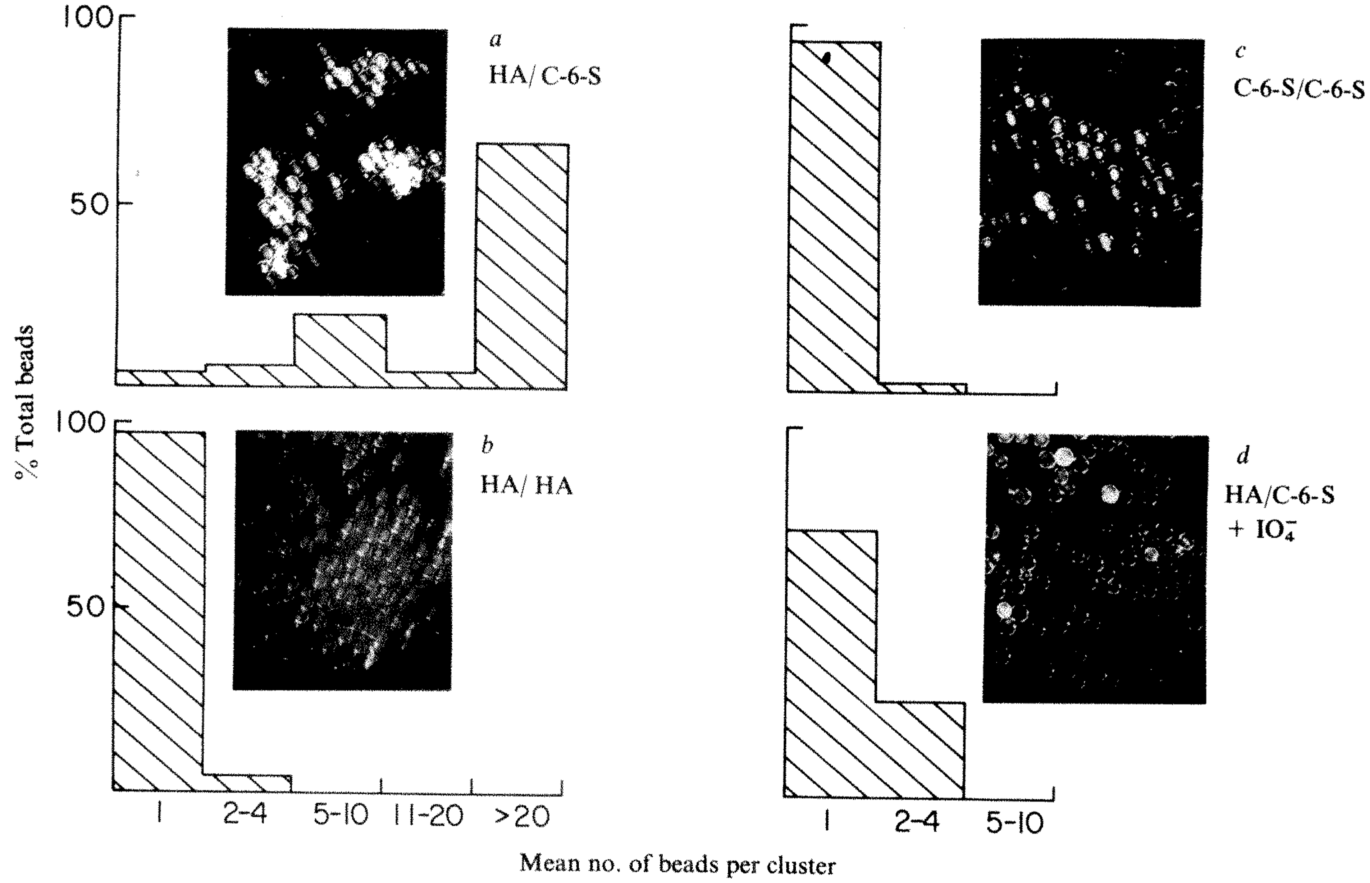

Fig. 1 The heterologous (*a*) and homologous (*b*, *c*) interaction of sulphonamide beads derivatised with either hyaluronic acid or chondroitin-6-sulphate. Agglutination assays were performed in 35 × 10 mm Falcon tissue culture dishes and initiated by adding 0.01 g of each of the desired bead types in 0.1 ml PBS (*p*H 7.4) to 2 ml of PBS at 4 °C. Approximately 30 μg of hyaluronic acid or 100 μg of chrondroitin 6-sulphate were derivatised to each mg of beads (dry weight). The dishes were agitated gently for 5 min at 4 °C. Agglutination was scored 12 h later by counting beads occurring in clusters or as single beads under a dissecting microscope. Dishes were shaken before counting the beads to separate loosely associated beads. The number of beads in each of the initial 0.1 ml aliquots was determined before the experiments were begun and the data are expressed as the mean number of beads per cluster. A total of 300 beads were counted in each agglutination assay. The experiment was repeated 20 times with different bead preparations and the results were quantitatively identical.

polymer solutions in the presence of a carbodiimide catalyst[12]. Details of the derivatisation and hydrolysis procedures are given in the legend to Table 1. The data in Table 1 show that the amount of HA and C-6-S derivatised to the beads is proportional to the amount of each compound present in solution during the incubation with carbodiimide. When HA and C-6-S were each present at 15 mg ml^{-1}, the highest concentration tested during the derivatisation incubation, more than 100 μg C-6-S became attached to 1 mg dry weight of the beads, whereas 25–30 μg hyaluronic acid became attached in identical conditions.

Agglutination assays with derivatised beads

Agglutination assays were performed as described in the legend to Fig. 1. Agglutination is pronounced when C-6-S beads are mixed with HA beads. Neither bead type shows any tendency towards self-agglutination. When HA beads are mixed with C-4-S beads, there is an agglutination reaction although it is approximately 40% as extensive as the reaction that occurs when HA beads and C-6-S beads are mixed. HA beads show only minimal agglutination when mixed with beads derivatised with heparin or dermatan sulphate. It is not clear whether the HA/CS interaction is specific for C-6-S and if this compound is contaminating our preparations of C-4-S, or whether hyaluronate will interact with either of the two chondroitins but with C-4-S to a lesser degree. The agglutination reactions described here were unaffected by temperature from 4° to 37 °C, calcium ion concentration of 0–20 mM, osmolarity of 0–300 mosM, and time of 5 min to 12 h.

Since the amount of sulphated glycosaminoglycan, other than C-6-S, bound to the beads was not measured, the decreased degree of agglutination may reflect a difference in concentration. There is no measurable agglutination among HA beads themselves or among beads subjected to the derivatisation procedures but without glycosaminoglycans. Hyaluronic acid beads initially agglutinate weakly with the underivatised beads but the interaction disperses within several hours.

C-6-S beads also show no self-agglutination, and they do not agglutinate underivatised beads or beads coated with other sulphated glycosaminoglycans. The absence of such homotypic agglutination can be seen in Fig. 1*b* and *c*. By contrast, the agglutination that occurs when these two bead types are mixed is shown in Fig. 1*a*. It is unlikely that the agglutination between the HA beads and the C-6-S beads reflects a nonspecific ionic attraction of charged polymers since neither bead type shows any tendency to self-agglutinate in the conditions tested here.

Since the glycosaminoglycans used in these experiments are 99.9% free of protein it is probable that the agglutination results from interactions between the carbohydrate chains of the hyaluronic acid and chondroitin sulphate. Further evidence for this conclusion can be seen in Fig. 2. The data in the left column of Fig. 2 show that the HA bead/C-6-S bead interaction is sensitive to mild periodate oxidation of either bead type. However, periodate oxidation of C-6-S has less effect on the HA/C-6-S interaction than oxidation of the hyaluronic acid polymer. This may reflect the relative resistance of C-6-S to periodate oxidation[19]. The right-hand column shows that HA bead/C-4-S bead interaction is abolished when C-4-S beads are treated with galactose oxidase. The lower right panel of Fig. 2

shows that the inhibition of agglutination by galactose oxidase can be partly reversed if the oxidised C-4-S beads are subsequently reduced with borohydride. C-4-S beads treated with galactose oxidase and 10–100 mM of the competitive substrate, galactose, retained their ability to agglutinate with HA beads. Heat-denatured galactose oxidase had no effect on the HA bead/C-4-S interaction and the active enzyme had no effect on HA beads. These results are consistent with the specificity of galactose oxidase for the C-6 hydroxyl group of galactosides and acetylated galactosaminides. Beads derivatised with oligosaccharide fragments of HA or C-6-S, prepared by digestion with testicular hyaluronidase and estimated to contain 8 residues by gel chromatography, agglutinated to a lesser extent than the beads derivatised with untreated polymers. Trypsin had no effect on HA bead/C-6-S bead interaction. In another experimental series, hyaluronate and chondroitin sulphate were subjected to mild base hydrolysis and reduction by sodium borohydride[22] to release residual protein. When these polymers were attached to beads, the HA bead/C-6-S bead agglutination occurred normally. These data, coupled with the relative absence of protein in the glycosaminoglycan preparations, suggest strongly that agglutination between beads containing hyaluronic acid and chondroitin sulphate represents a carbohydrate–carbohydrate interaction.

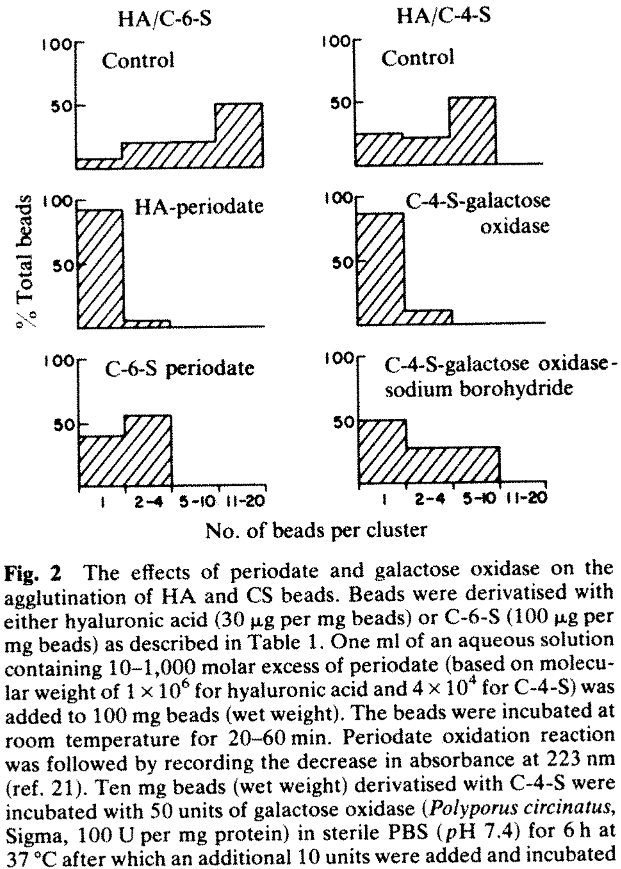

Fig. 2 The effects of periodate and galactose oxidase on the agglutination of HA and CS beads. Beads were derivatised with either hyaluronic acid (30 µg per mg beads) or C-6-S (100 µg per mg beads) as described in Table 1. One ml of an aqueous solution containing 10–1,000 molar excess of periodate (based on molecular weight of 1×10^6 for hyaluronic acid and 4×10^4 for C-4-S) was added to 100 mg beads (wet weight). The beads were incubated at room temperature for 20–60 min. Periodate oxidation reaction was followed by recording the decrease in absorbance at 223 nm (ref. 21). Ten mg beads (wet weight) derivatised with C-4-S were incubated with 50 units of galactose oxidase (*Polyporus circinatus*, Sigma, 100 U per mg protein) in sterile PBS (*p*H 7.4) for 6 h at 37 °C after which an additional 10 units were added and incubated for another 12 h. The enzyme solution was decanted and beads were washed in sterile PBS twice and heated at 100 °C for 15 min to inactivate the residual enzyme. One half of the enzyme-treated beads were subsequently incubated with 10 mM sodium borohydride for 10 h at room temperature. Unreacted sodium borohydride was then destroyed by careful addition of 0.1 M acetic acid. Oxidised and reduced C-4-S beads were again washed several times and then assayed for their ability to agglutinate with HA-beads as described in Fig. 1. Incubation of C-4-S beads with galactose oxidase and the competitive substrate, galactose, with boiled enzyme or with sodium borohydride, glycerol or acetic acid did not alter agglutination with HA beads. This experiment, like those reported in Figs 3 and 4, was repeated four times with different bead preparations and the results was quantitatively identical. A total of 300 beads were counted for each assay.

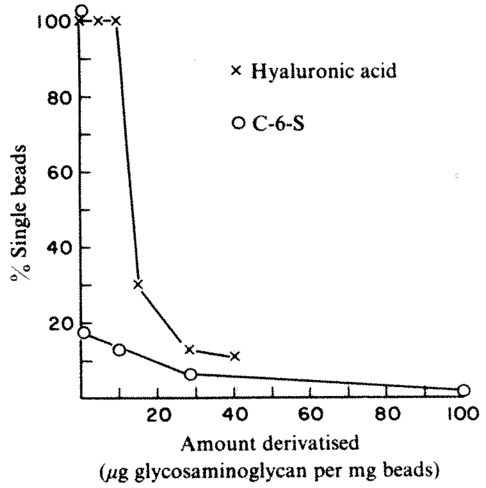

Fig. 3 The agglutination of HA beads with C-6-S beads as a function of the amount of polymer derivitised to beads. Beads were incubated with 0.1 to 15 mg per 100 mg beads of either HA or C-6-S together with 5 times (w/w) that amount of carbodiimide as described in Table 1. Aliquots of beads derivitised with 0–100 µg C-6-S per mg bead (dry weight) were mixed with HA beads (30 µg HA per mg bead, dry weight). Similarly, aliquots of beads derivatised with 0–30 µg HA per mg bead were mixed with C-6-S beads (100 µg C-6-S per mg bead). Beads were shaken gently for 5 min and then counted as described for the agglutination assay in Fig. 1. Agglutination was expressed as per cent single beads. A total of 300 beads were counted for each assay.

The agglutination between HA beads and C-6-S beads is dependent on the amount of each polymer bound to the beads. In Fig. 3, agglutination is determined as a function of the amount of HA and C-6-S derivatised to the beads. C-6-S beads with as little as 0.1 µg polymer per mg (dry weight) bead are able to agglutinate maximally with HA beads containing 30 µg HA per mg bead. Hyaluronic acid beads, however, require at least 30 µg HA per mg bead to agglutinate maximally with beads containing 100 µg C-6-S per mg beads.

The agglutination of the C-6-S beads with the HA beads is sensitive to either hyaluronic acid or C-6-S in solution. The standard agglutination reaction was performed with increasing amounts of soluble glycosaminoglycans added to the incubation mixture (Fig. 4). The agglutination appears to be more sensitive to hyaluronic acid since as little as 5 µg ml^{-1} of this polymer is able to inhibit the agglutination of the two bead types. C-6-S in solution is not an effective inhibitor below concentrations of about 50 µg ml^{-1}. After the addition of C-6-S or hyaluronic acid, the bead clusters begin to disperse so that within 1–2 h beads are no longer agglutinated. Neither oligosaccharide fragments nor monosaccharides of hyaluronic acid or C-6-S were able to inhibit bead agglutination.

Discussion

Beads derivatised with hyaluronic acid spontaneously agglutinate beads derivatised with C-6-S when the two bead types are mixed in PBS or in distilled H_2O. The agglutination occurs within minutes at temperatures between 4 °C and 37 °C and remains stable for as long as one day. Hyaluronic acid beads also interact with beads coated with other sulphated glycosaminoglycans such as C-4-S, heparin and dermatan sulphate although to a lesser extent. Homologous interactions among beads coated with one glycosaminoglycan do not occur.

The interactions described here were obtained using glycosaminoglycan preparations containing less than 0.1% protein and this fact alone suggests that the agglutination reflects an interactive specificity between the carbohydrate chains of hyaluronic acid and chondroitin sulphate. This conclusion is further supported by other observations. The HA/sulphated glycosaminoglycan interaction is sensitive to mild periodate oxidation, to treatment with testicular hyaluronidase, which hydrolyses the carbohydrate chain of the polymers to oligosaccharides, and to galactose oxidase treatment of C-4-S. The interaction was not disrupted by trypsin treatment nor by mild base hydrolysis of the polymers in conditions reported to release protein[22]. Although periodate can affect protein, the conditions used here minimise protein oxidation[23]. The galactose oxidase used in this study was not a homogeneous enzyme and the preparation may have contained other degradative enzymes that inhibited the agglutination between the HA beads and the C-4-S beads. Three experiments suggest, however, that the inhibition was due to the action of galactose oxidase and not to impurities. First, the enzyme preparation had no effect on HA-beads. Second, the inhibition of agglutination that resulted when C-4-S beads were treated with galactose oxidase could be partly overcome by subsequent treatment with sodium borohydride. Third, galactose oxidase in the presence of the competitive substrate, galactose, had no effect on C-4-S beads. These results indicate that the galactose oxidase preparation was interacting with C-4-S polymer in a manner consistent with its catalytic specificity. It seems very unlikely, therefore, that the agglutination described in this report is mediated by protein. The most reasonable explanation is that the agglutination reflects an interaction between the carbohydrate chains of chondroitin sulphate and hyaluronic acid.

In plants and bacteria, the binding between certain homologous and heterologous carbohydrate chains is known to occur. Cellulose, the most abundant biological molecule, consists of lengthwise aggregates of glucose polymers. Also, galactomannans, glucomannans, arabinoxylans and celluloses can bind to agarose and K-carageenans. The plant pathogen *Xanthomonas* may bind to host cellulose via its galactomannan chains[3]. Suggested mechanisms for such binding between carbohydrate chains include aggregated helices, interactions along specific regions to form 'egg-box' structures, or combinations of the two[3].

The mechanism of binding between the hyaluronate and sulphated glycosaminoglycan chains described here is entirely unknown. Homologous chains of glycosaminoglycans are known to aggregate[25] and to form helices in solution[26–28]. The homologous interaction seems to differ from the HA bead/CS bead agglutination described here since homologous beads did not agglutinate with one another. The ability of soluble polymers to inhibit and to reverse the agglutination of the derivatised beads suggests that the binding may be an equilibrium with an appreciable dissociation rate.

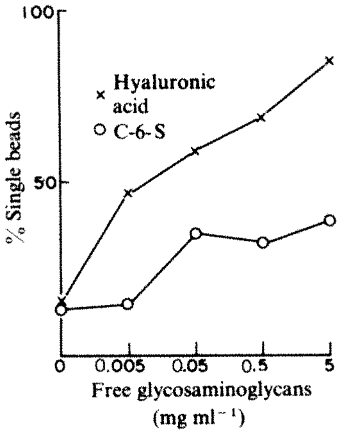

Fig. 4 The effect of soluble glycosaminoglycans on the agglutination of HA and C-6-S beads. Solutions of glycosaminoglycans were prepared by dissolving from 5 μg to 5 mg of either HA or C-6-S polymer and adding the solutions to agglutinated C-6-S and HA beads. Beads and the glycosaminoglycan solutions were incubated at 4 °C for 10 h. Beads were then counted and the extent of agglutination expressed as per cent single beads. Solutions containing monosaccharide or oligosaccharide fragments (approximately 4–8 residues) had no effect on the interaction between HA and C-6-S beads. Oligosaccharide fragments were prepared by digestion with testicular hyaluronidase (Worthington 0.4 mg ml^{-1}) in 0.1 M sodium phosphate buffer containing 0.15 M sodium chloride at *p*H 5.3[24]. The molecular weight was estimated by chromatography on Sephadex G-200 column in SDS buffer. Sephadex fractions were assayed for carbohydrate with phenol sulphuric acid[15].

There is considerable evidence to show that hyaluronate can interact with proteoglycan. Muir and her colleagues have shown, for example[29], that the elution profile of disaggregated, cartilage proteoglycans on Sepharose 2B is changed markedly by the addition of as little as 0.625% hyaluronate. The hyaluronate and the proteoglycan elute together earlier than either would have alone. It is not proposed here that the carbohydrate–carbohydrate interaction is the mechanism for such observations—merely that it is another mechanism for specific interactions among proteoglycan components.

There are at least two reasons that could account for the failure of previous experiments to detect a heterologous interaction between the carbohydrate chains of HA and CS. First, no obvious precipitation occurs when the soluble compounds are mixed. This may indicate that the number of saccharide chains involved in complex formation in solution is small. Second, when hyaluronate/proteoglycan interactions are under examination, there is no reason to believe that the heterologous interaction between the carbohydrate chains would interfere with the hyaluronate/protein interaction. The avidity with which hyaluronate binds to CS may, in fact, be much less than that with which it binds to the protein of the proteoglycans. These comparisons will require further experimentation for the precise determinations of true binding constants.

Much research has implicated the glycosaminoglycans, particularly hyaluronate and chondroitin sulphate, in cell–cell adhesion[1,5], cell–substrate adhesion[6,12], organogenesis[7], and differentiation[8]. In many embryos, the control of deposition, degradation, and the spatial arrangements of the glycosaminoglycans suggest that they may have a key role in morphogenesis. Precisely how these polymers can effect adhesive behaviour and morphogenetic phenomena is not known but a specific binding between heterologous, carbohydrate chains could represent one mechanism.

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LETTERS

Distribution of polarised radio emission in M31

Rainer Beck, Elly M. Berkhuijsen & Richard Wielebinski

Max-Planck-Institut für Radioastronomie, Bonn, FRG

Observations yielding the distribution of large-scale magnetic fields in nearby spiral galaxies are still extremely scarce. Optical polarisation of starlight has been observed in the disks of M31, M51, M81 and some other spiral galaxies[1–3], but these measurements are too few to reveal the general distribution and direction of the magnetic fields. Segalovitz *et al.*[4] observed the distribution of the polarised radio emission in M51 at λ21 cm and λ6 cm with sufficient linear resolution (2.6 × 3.6 kpc) and sensitivity to conclude that the magnetic field in M51 is predominantly directed along the spiral arms. The first results of detailed polarisation measurements of the emission of M31 at λ11 cm (ref. 5) indicated that in the southern part of the bright ring of radio emission the magnetic field is generally aligned along the ring. We show here that this early conclusion seems to hold for the whole of the ring.

The observations were carried out with the 100-m telescope of the Max-Planck-Institut für Radioastronomie at Effelsberg between 1977 and 1978. The dual-channel correlation polarimeter in the secondary focus operated at 2,700 MHz (system temperature, 100 K; bandwidth, 80 MHz; observed half-power beamwidth, 4.4 arc min). The flux densities and polarisation characteristics of the calibration sources 3C48, 3C138 and 3C147 were taken from Baars *et al.*[6] and Wardle and Kronberg[7]. The ratio of main beam brightness temperature T_b(K) to the flux density S(Jy) was found to be 2.3 ± 0.1. 3C147 was used to determine the spurious instrumental polarisation, which was found to be highest at 2 arc min distance from the source with ~0.5% of the total emission.

Figure 1 shows the λ11.1 cm polarisation map of M31 superimposed on an optical picture. The map covers the area $-81 < \lambda_A < +81$ arc min, $-45 < \beta_A < +45$ arc min, where the coordinates λ_A and β_A lie along the major and minor axis, respectively. The area was observed in three parts, each overlapping by 10 arc min in λ_A. The northern part and the central part were scanned both in λ_A and β_A (~10 times each), while the southern part was scanned only in β_A (~20 times). For the northern and the central parts the averages of the maps in λ_A and of the maps in β_A were combined using a technique for optimising the base levels developed by C. J. Salter. By applying this technique to the maps in Stokes parameters Q and U separately, any polarised foreground emission varying linearly across the map is eliminated. (Faraday rotation in the foreground is not affected by this procedure.)

The polarisation temperatures derived from $(Q^2+U^2)^{1/2}$ suffer from a systematic positive bias due to noise. All values in Fig. 1 have, therefore, been corrected as proposed by Wardle and Kronberg[7]. The r.m.s. noise σ in the final map is 3 mK in main beam polarisation brightness temperature T_b^p.

A map of the total intensity of the area at λ11.1 cm was obtained from the total power channels of the same observations (Fig. 2); the r.m.s. noise is 5 mK in main beam brightness temperature T_b. This new total power map does not differ significantly from the map published by Berkhuijsen and Wielebinski[8], but it has a slightly better angular resolution and lower r.m.s. noise. The new map extends further in β_A so that the

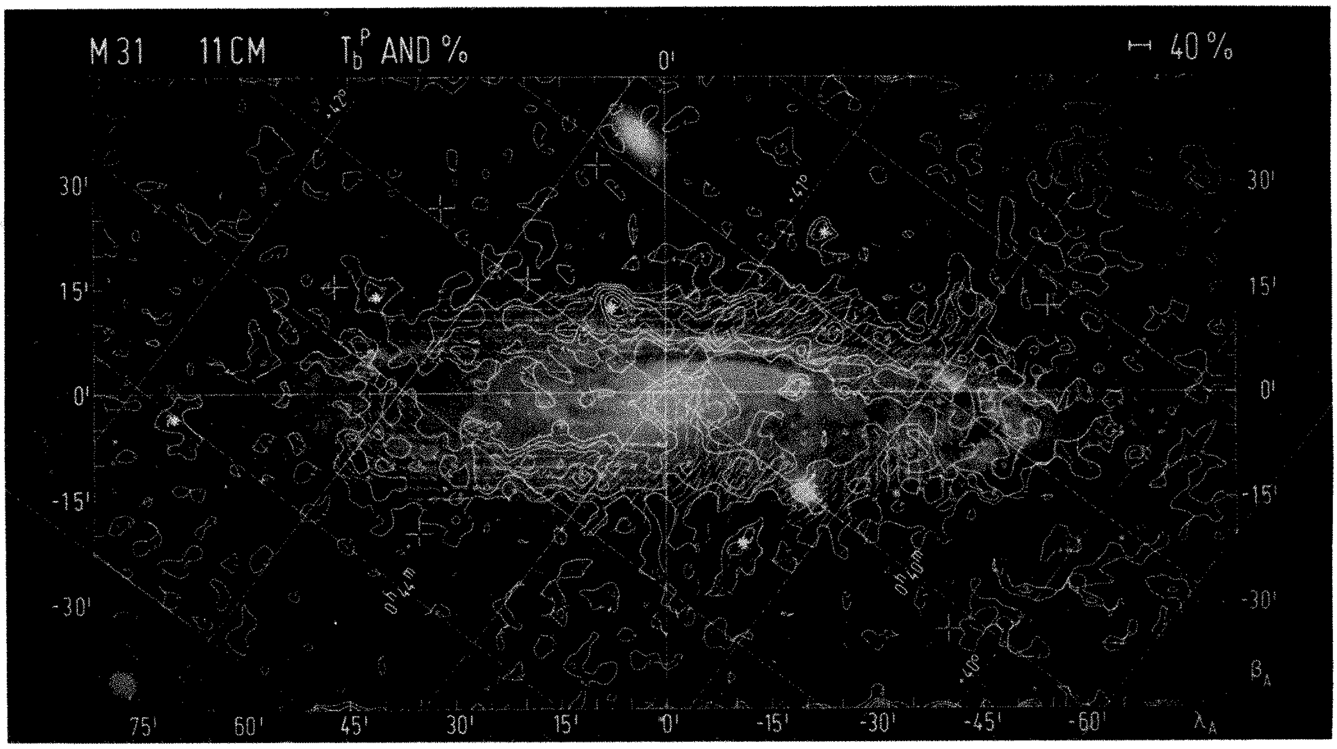

Fig. 1 Contour map of main beam polarisation brightness temperature T_b^p at λ11.1 cm superimposed on an optical picture of M31 (photograph from Hale Observatories). The first contour level is 4 mK ($=1.3\sigma$) in T_b^p; the contour interval is 4 mK. The length of the vectors is proportional to the percentage polarisation p which was only computed if $T_b^p > 3$ mK and $T_b > 15$ mK. Asterisks show the position of polarised point sources 5C3.73, 107, 120, 126 and 163.

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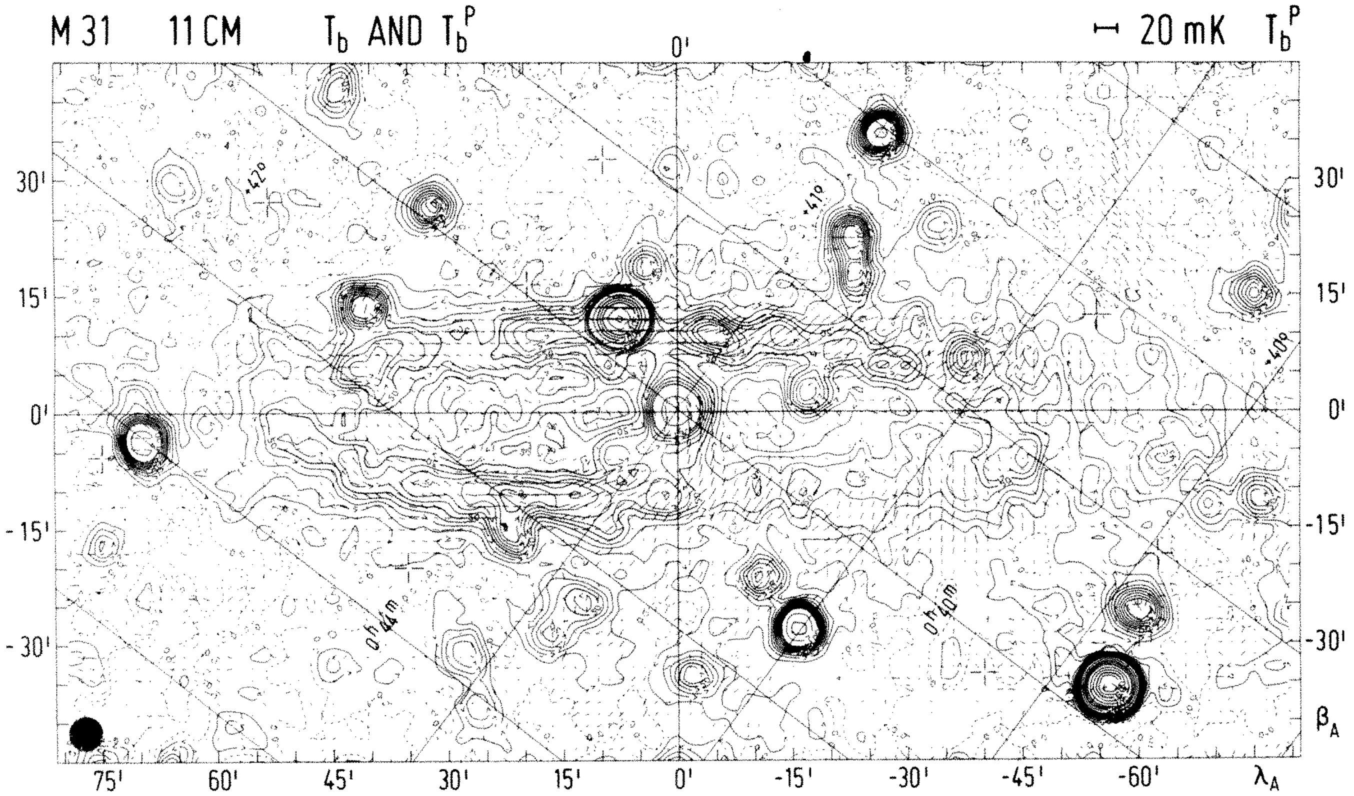

Fig. 2 Contour map of the main beam brightness temperature T_b. Contour levels are: 0, 10, ..., 100, 150, ..., 300, 400, ..., 800 mK. The length of the vectors is proportional to the main beam polarisation brightness temperature T_b^P.

zero level could be determined with more confidence. The zero level in Fig. 2 is higher by 5 ± 3 mK compared with the old map.

The total power map of Fig. 2 was used to compute the degrees of polarisation as shown by the vectors in Fig. 1.

Figure 1 contains the optically brightest parts of M31 out to a radius $R = 16$ kpc along the major axis adopting a distance to M31 of 690 kpc[9]. The resolution of 4.4 arc min corresponds to 0.9×3.2 kpc in the plane of M31 (inclination $i = 74°$; $i = 0°$ is face-on). This is the finest linear resolution used to date for observing the distribution of the polarised radio emission in a nearby spiral galaxy. Because the width of the arms on the major axis is ~1.5 kpc (ref. 10) this resolution is small enough to study the large-scale magnetic field in some detail.

The outstanding feature of Fig. 1 is the concentration of the polarised emission in a ring around the centre. This ring approximately coincides with the optically brightest arms (nos 4 and 5 in the notation of Baade[11]) which contain most of the H II regions, as well as with the rings of high intensity seen at λ 11 cm in total power (Fig. 2 and ref. 10) and in the neutral hydrogen line[9].

The radial distribution of the polarised intensity is shown in Fig. 3. In both the northern and in the southern half of M31 the bright ring extends from $R = 5$ to $R = 12$ kpc reaching a maximum intensity of $T_b^P \sim 8$ mK at $R = 8$ kpc. At some places in the ring ($\lambda_A = +20$ arc min, $\beta_A = -10$ arc min; $\lambda_A = -10$ arc min, $\beta_A = +10$ arc min) local maxima of $T_b^P \cong 18$ mK occur. Maxima due to polarised point sources are indicated in Fig. 1; the source with the highest polarised flux density (7 mJy corresponding to a degree of polarisation of 3%) is 5C3.107 at $\lambda_A = +7$ arc min, $\beta_A = +12$ arc min. In the ring several minima are seen coincident with large H II regions. At these positions the H II regions are probably in front of the synchrotron emission regions and depolarise the radiation.

In the north-east ($\lambda_A > 0$ arc min, $\beta_A < 0$ arc min), the north-west ($\lambda_A > 0$ arc min, $\beta_A > 0$ arc min), and the south-west ($\lambda_A < 0$ arc min, $\beta_A > 0$ arc min) the lines of maximum intensity (ridge lines) in polarisation as well as in total power (Fig. 2 and ref. 10) and H I (ref. 9) generally coincide with dust lanes. In the south-east ($\lambda_A < 0$ arc min, $\beta_A < 0$ arc min), however, the ridge line in total power is less clearly coincident with dust lanes, and the ridge line in polarisation is shifted outwards with respect to the optical spiral arm.

In the north-west, outside the bright ring, several weak maxima in T_b^P seem to line up along arm 5; all of these maxima coincide with dust regions. Conversely, the polarised feature at $\lambda_A = -60$ arc min, $\beta_A = +15$ to $+35$ arc min does not seem to be related to M31; this is probably a foreground irregularity within 500 pc of the Sun[5].

Close to the centre of M31 an area with $T_b^P > 12$ mK is seen which seems to connect with the bright ring to the east but not to the west. This emission might originate in a region extending up to $z \cong 2$ kpc from the nucleus. If the western side were the closer to Earth, most of this emission would be seen in front of the plane to the east, while most would be depolarised by the plane to the west.

Figure 1 shows a well ordered pattern in the distribution of the polarisation angles. Near the southern major axis ($\lambda_A < -30$ arc min) the vectors are predominantly directed perpendicular to the spiral arm to within ~30°. This orientation continues throughout the south-east quadrant, but near $\lambda_A = +5$ arc min, $\beta_A = -10$ arc min a clockwise rotation of the vectors of ~90° changes their preferential direction to along the spiral arm. In the south-west quadrant a similar clockwise rotation of the vectors occurs between $\lambda_A = -30$ and -10 arc min; the vectors remain orientated along the arm throughout the north-west quadrant and also follow the turn of the arm near the northern major axis.

In the absence of Faraday rotation the vectors are perpendicular to the projection of the magnetic field onto the plane of the sky. The orientation of the vectors perpendicular to the arm in the south-west and south-east then implies that the magnetic field is directed along the arms. Between $\lambda_A = -45$ and -30 arc min the angle ϕ between the arm and the line of sight varies between 16° and 60°, in which interval the rotation measure (RM) should increase by a factor of 2. As the perpendicular orientation of the vectors to the arm is maintained over

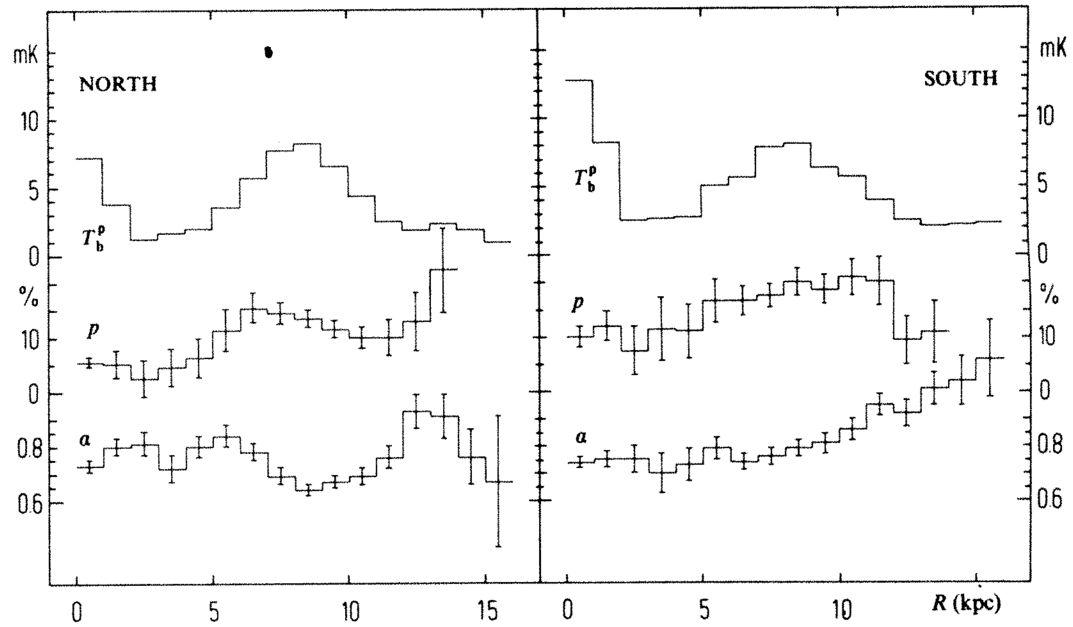

Fig. 3 Dependence of T_b^p, p and $\alpha_{408}^{2,700}$, as determined in circular rings in the plane of M31, on the distance R to the centre, shown for the north ($\lambda_A > 0$ arc min) and the south ($\lambda_A < 0$ arc min) separately.

this interval, an upper limit to the internal Faraday rotation on the major axis can be set of $\Delta\theta < 30°$, or RM < 42 rad m^{-2}. With a field component along the line of sight of 2 μG (ref. 12) and a path length through the arm on the major axis of 200 pc/cos 74° the mean density of the thermal electrons is found to be $N_e < 0.04$ cm^{-3}. This is comparable to the mean electron density of ~0.03 cm^{-3} derived by Ables and Manchester[13] for the local region in our Galaxy.

The clockwise rotation of ~90° observed all over the northern part of M31 is believed to originate in the galactic foreground because: (1) the rotation is independent of ϕ; and (2) it is unlikely that N_e in the entire northern part of M31 is at least three times higher than in the southern part because the north/south ratio of the thermal fraction is only ~1.5 (see Table 1). A rotation of −90° corresponds to RM $= -127$ rad m^{-2} which is in the range of rotation measures found by Simard-Normandin and Kronberg[14] near M31.

Figure 1 shows that the average intensity of the polarised emission in the ring exceeds 10 mK near the minor axis but is only ~3 mK near the major axis. If the velocity field of the relativistic electrons is isotropic, such an effect is to be expected in the case of a magnetic field preferentially orientated along the bright ring: on the minor axis we are then looking perpendicular to the field ($\phi = 90°$) and see high T_b^p, while on the major axis we are looking nearly along the field ($\phi = 16°$) and find low T_b^p. Faraday effects would enhance this difference.

Thus both the variation of T_b^p in the ring of emission and the orientation of the vectors indicate that the magnetic field is preferentially aligned along the arms that form the ring.

In Fig. 1 the lengths of the vectors are proportional to the degree of polarisation, $p = T_b^p/T_b$, where T_b is the sum of thermal and nonthermal emission. The nucleus of M31 is ~7% polarised. The degree of polarisation is generally higher in the south than in the north. The maximum percentage observed is $p = 42\%$ at $\lambda_A, \beta_A = -10, +12$ and $-24, +12$ arc min in the south-west, and $\lambda_A, \beta_A = -12, -15$ and $-20, -15$ arc min in the south-east; in the north-east the highest percentage is $p = 37\%$ at $\lambda_A, \beta_A = +27, -5$ arc min. Several minima in the degree of polarisation are coincident with complexes of H II regions seen on the map of Pellet *et al.*[15] (that is, $\lambda_A, \beta_A = +40, +6$ arc min; $\lambda_A, \beta_A = +12, -12$ arc min; $\lambda_A, \beta_A = -45, -9$ arc min) which may cause depolarisation.

To study the dependence of the degree of polarisation on distance to the centre, the average value of p was determined in circular rings in the plane of M31 so that the average of the angle ϕ is the same for every ring. The variation of p with R is shown in Fig. 3 for north and south separately. The variation of the mean spectral index $\alpha_{408}^{2,700}$ (ref. 12) is also shown. In the north, p increases from 5% near the centre to 15% at $R \sim 7$ kpc on the inner side of the bright ring, and then decreases in the ring to 10% at $R \sim 11$ kpc. The spectral index also decreases markedly in the bright ring. This variation may be due to an increase of the thermal fraction in the ring, which will cause a decrease in p by a larger thermal contribution to T_b and an increase in Faraday depolarisation. In the south the situation is different: p and $\alpha_{408}^{2,700}$ are higher than in the north and show a constant increase with R across the bright ring.

Average values out to $R = 16$ kpc of p, $\alpha_{408}^{2,700}$, the thermal fraction f_{th} and the degree of polarisation corrected for the thermal contribution, p_n, are collected in Table 1 (ref. 12). The higher degree of polarisation in the southern half is only partly due to lower thermal fraction: there remains an excess of the polarised emission while there is no asymmetry in the total nonthermal emission.

Locally p_n will be much higher than the average values: at positions where $p = 42\%$, clearly $p_n > 42\%$. In the north-east similar high values of p_n are found if $f_{th} \sim 70\%$. In the case of a uniform field the intrinsic degree of polarisation of the nonthermal emission for $\alpha_n = 0.85$ is $p_i = 73.5\%$. Assuming that values of $p_n > 42\%$ are not affected by Faraday depolarisation, but that the difference between p_i and p_n is entirely due to irregularities of the magnetic field in the emission regions along the line of sight, the ratio of random field to uniform field is <0.9, corresponding to a degree of uniformity of >50% on the scale of the beamwidth[16]. With the model of Segalovitz *et al.*[4] a degree of uniformity >40% and a ratio of random field/uniform field <1.5 is derived. Thus the degree of uniformity of the magnetic field in M31 on a scale of 0.9 × 3.2 kpc is about three times higher than that found in M51 on a scale of 2.6 × 3.6 kpc (ref. 4). As the width and the separation of the arms as seen in the plane of the sky is comparable in these galaxies, the difference in the degree of uniformity of the field may be caused by a larger depolarisation across the approximately three times larger scale of the beam area used for M51.

Table 1 Average values for $R \leq 16$ kpc

Area	$\bar{p}$ (%)	$\bar{\alpha}_{408}^{2,700}$	$\overline{f_{th}}$ (%)	$\overline{p_n}$ (%)
Total	14.6 ± 1.5	0.76 ± .05	30 ± 5	21 ± 3
North	11.7 ± 1.4	0.71 ± .05	35 ± 6	18 ± 3
South	18.3 ± 1.9	0.82 ± .05	24 ± 4	24 ± 3

An estimate of the Faraday depolarisation and a study of the intrinsic polarisation angles in the bright ring of M31 can be made when our observations at λ 18 cm (underway) and λ 6 cm (planned) are complete.

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Peculiarities of binary galaxies

Nigel Sharp & Bernard Jones

Institute of Astronomy, Madingley Road, Cambridge, UK

A recent analysis of a sample of close binary galaxies[1] by White and Valdes[2] has revealed some peculiar features: in particular, they tend to be brighter than field galaxies. Binary galaxies are important because the relative simplicity of the two-body problem in dynamics should make it possible to obtain good estimates of the masses of paired galaxies. This would help in determining whether there are massive haloes of dark, unseen matter, and in the problem area of galaxy formation. Much effort has therefore been expended (ref. 3 and refs therein), but conclusions about the existence of massive haloes and about the mass-to-light ratios have varied. Because we do not have complete information, various statistical assumptions must be made in these analyses. Detailed arguments[2] suggest that one of these assumptions has been in error in previous work. We present a test which supports this claim. (The sample used here and in ref. 2 seems to be the only set of paired galaxies with quantitative selection criteria and velocity information for an unbiased subsample.) Furthermore, it was shown[2] that binary galaxies are drawn from a luminosity function which has a characteristic luminosity brighter than that for field galaxies by almost one whole magnitude. We discuss one of the possible explanations and some suggestive evidence.

The basic observational information available includes the apparent magnitude of the two galaxies, their redshifts, and their angular separation on the sky, θ. The (probably reasonable) assumption that the centre of mass of the system partakes of the Hubble flow enables these data to be converted to absolute luminosities, a line-of-sight velocity difference, and a projected rectilinear separation r_p (see ref. 3). Further analysis of a single pair would require knowledge of the angle between the true separation vector and the line-of-sight, ϕ, the orbital eccentricity, and the orbital phase. Since we do not have these data, we must make assumptions about them. Such assumptions are usually statistical in nature, because samples usually comprise many pairs. Thus, for example, one can assume a distribution of eccentricities in the sample. This seems to indicate the need for low eccentricity, but is apparently not crucial to mass determinations[3].

Another important assumption is the distribution of the angle ϕ, which relates the true separation r to the projected separation r_p by $r_p = r \sin \phi$. Previous analyses[3–5] have used a distribution $N(\phi)\,d\phi \propto (\sin\phi)^\alpha\, d\phi$ with an exponent $\alpha = 0.5$, derived from the data. The recent analysis of White and Valdes[2], however, uses the covariance function to derive $\alpha = -(2-\gamma)$ for a two-point function $\xi(r) = (r_0/r)^\gamma$. The accepted value for γ of 1.8 thus leads to $\alpha = -0.2$. It seems that the earlier authors erroneously assumed that pairs isolated from correlated objects have the orientation distribution appropriate to pairs isolated from an uncorrelated population. The change from $\alpha = 0.5$ to $\alpha = -0.2$ increases mass determinations, made using Turner's method[3], by about 40%, and can affect rank–sum techniques[4] by as much as a factor two[2] (although the use of overlapping bins in this analysis may have introduced other sources of uncertainty). It is therefore very important to decide which exponent is correct.

It was suggested by D. Lynden-Bell (personal communication) that it should be possible to use a test analogous to the V/V_{max} test used to study the space distribution of quasars[6]. For the present sample, we consider the angular separation θ and the maximum separation that the pair could have had and still be included in the sample, θ_{max}. This latter angle is either one-fifth of the distance to the third galaxy nearest the pair, or 8 arc min, whichever is the smaller (see the selection criteria of ref. 1). Figure 1 shows a plot θ/θ_{max} against θ_{max} for the full sample of 156 pairs[1]. (Because the values for θ are the precise measurements of Turner, whilst the values for θ_{max} come from neighbour calculations using the positions given in the Zwicky catalogue, some ratios are greater than one. This increases the scatter without introducing any significant problems).

Assuming a power-law distribution as above gives $n(\theta)d\theta \propto \theta^{-\alpha}d\theta$, which then predicts that the average value $\langle\theta/\theta_{max}\rangle$ is $[(1+\alpha) \pm N^{-1/2}(1+\alpha)^{1/2}(3+\alpha)^{-1/2}]/(2+\alpha)$ for a sample size of N. For 156 pairs, the data give $\langle\theta/\theta_{max}\rangle = 0.52 \pm 0.02$; the predictions are 0.33 ± 0.02 ($\alpha = 0.5$) and 0.55 ± 0.02 ($\alpha = -0.2$). This suggests that $\alpha = -0.1 \pm 0.1$. Various subsamples have been used in different works based on binary galaxies[2,3,7,8], and there is no obvious systematic variation in the values of $\langle\theta/\theta_{max}\rangle$ for them. In addition, there does not seem to be any clear dependence on θ_{max} for any of the sets. The recent argument is thus clearly supported.

The second puzzling aspect is the apparently increased luminosity of paired galaxies. An obvious explanation is that the interaction of the two galaxies induces star formation and thus

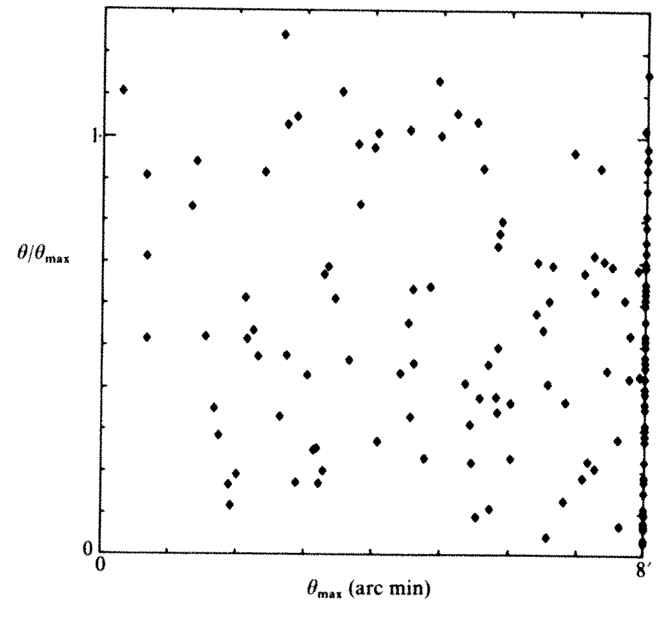

Fig. 1 The ratio θ/θ_{max} plotted against θ_{max} for the 156 pairs of ref. 1.

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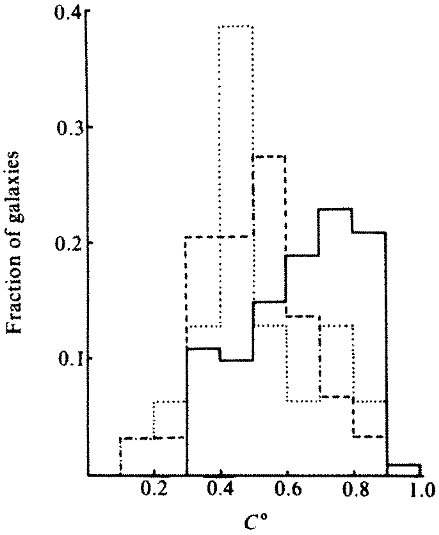

Fig. 2 The distribution of the absorption corrected, integrated B–V colour index $C°$. solid line: 100 randomly chosen non-elliptical galaxies. Dashed line: close spiral–spiral pairs. Dotted line: wide spiral–spiral pairs.

makes them brighter. This idea is supported by work on normal and peculiar galaxies[9]. As the method by which star formation might be enhanced is not clear, possible correlations between separation, morphological type, and/or luminosity excess, are not well predicted.

An excellent check would be to consider the colours of the binary galaxies, since one would expect that sufficient star formation to increase the luminosity to the required extent would make the galaxies quite distinctly bluer[9]. These data are not available for the Turner sample, but some suggestive evidence comes from a survey (B. J., unpublished) based on the data of Holmberg[10], split into 'wide' pairs, 'close' pairs, and the field. By 'close' is meant a pair whose separation is of the order of or less than the sum of the face-on diameters of the components. 'Wide' comprises systems which are essentially not touching.

Figure 2 compares three histograms showing the distribution of the absorption-corrected integrated B–V colour index $C°$. The solid line is for 100 randomly chosen non-elliptical galaxies; the dashed line is for close spiral-spiral pairs; the dotted line is for wide spiral–spiral pairs. The mean colour index for the three classes goes from 0.64 (field) to 0.50 (wide) and to 0.49 (close), with r.m.s. deviations of 0.16, 0.17 and 0.16 respectively. In addition, it is clear that the distributions for the paired galaxies are skewed to the blue of the 'ordinary' galaxies.

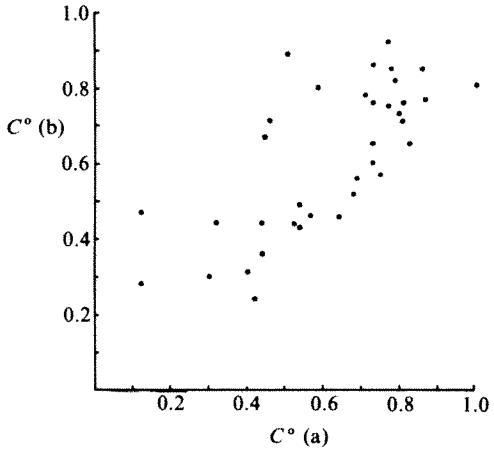

Fig. 3 The correlation between the colour index of the brighter (or larger) galaxy, $C°$(a), and that of its companion, $C°$(b).

That this colour change is due to interaction is strongly supported by the correlation between the colours of the two components. In Fig. 3 the colour index for the brighter (or larger) galaxy, $C°$(a), is plotted against that of its companion, $C°$(b), for the spiral–spiral pairs. The correlation coefficient is 0.7: red (blue) galaxies tend to have red (blue) companions. (There is also a suggestive correlation of morphological type[5].)

Let L_B and L_V be the B and V luminosities of a galaxy before interaction, and let l_B and l_V be the contributions from stars formed as a consequence of the interaction. Then $L_V/L_B = 10^{0.4C}$ and $l_V/l_B = 10^{0.4C_*}$, where C is the colour index of the pre-interaction galaxy and C_* is the mean colour index of the newly created stars. The colour change is than

$$\Delta C = -2.5\log_{10}\left\{\frac{1+10^{0.4(C-C_*)}l_V/L_V}{1+l_V/L_V}\right\} \qquad (1)$$

and the associated magnitude changes are $\Delta M_V = -2.5\log_{10}(1+l_V/L_V)$ and $\Delta M_B = \Delta M_V + \Delta C$. Figure 4 shows a few curves of $-\Delta C$ plotted against l_V/L_V, labelled by their values of $C - C_*$. The upper scale gives $(-\Delta M_V)$, and some curves of constant ΔM_B are also shown.

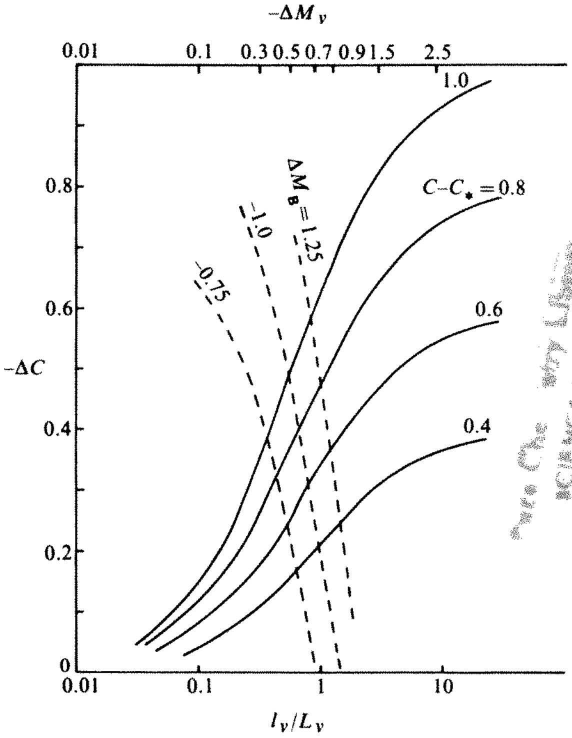

Fig. 4 The relation between the colour change ΔC and the magnitude changes ΔM_V and ΔM_B, for different values of $C - C_*$ (see equation (1)).

Figures 2 and 3 here, and Fig. 1 of ref. 9, suggest that a colour change of about 0.3–0.5 mag is required. The value of C_* to take is given roughly by the time elapsed since the interaction: choose the stars that spend that amount of time on the main sequence. (The finer details depend on the mass function of the stars.) If the galaxies interacted between 10^8 and 10^9 yr ago, C_* is in the range -0.15 to $+0.15$. An initial galaxy of C about 0.7 therefore has $C - C_*$ in the range 0.55 to 0.85. Figure 4 then indicates a value for ΔM_V of 0.5 to 0.8 mag, and for ΔM_B of 0.75 to 1.25 mag.

Although the samples are selected differently[1,10], the same physical processes should occur in both. In addition, it is not certain which stage in a binary galaxy's life is most propitious for induced star formation and the ages of the binary systems are difficult to determine. Although the orbital times for the Turner sample are so short that significant interaction (of a dynamical sort at least) seems inevitable[7,11], it also seems that the disk galaxy pairs were probably not dynamically isolated from other galaxies at the time of their formation[8]. This makes consideration of the induced star formation yet more complex.

However, the evidence presented here shows that significant colour changes are induced by interaction, and that these are of such a magnitude and take place over such a time scale that the B magnitude of a galaxy would increase by about one magnitude, as required by White and Valdes[2]. To confirm this, better data are required—particularly a detailed study of the colours of the Turner pairs.

Note added in proof: The extension of the covariance function to small scales[12] adds more weight to the arguments here and in ref. 2.

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Spectral evidence for a carbonaceous chondrite surface composition on Deimos

Kevin D. Pang

Planetary Science Institute, 283 South Lake Avenue, Suite 218,
Pasadena, California 91101

Jack W. Rhoads, Arthur L. Lane & Joseph M. Ajello

Jet Propulsion Laboratory, California Institute of Technology,
Pasadena, California 91103

The good match between the reflectance spectra of Phobos and Ceres, an asteroid inferred to have a composition analogous to carbonaceous chondrites because of its low density, ~2.2g cm^{-3} (ref. 1), and spectral signatures that include a water of hydration band centred at 3.0 μm (refs 2, 3), has led to the conclusion[4] that the surface composition of the inner martian satellite is similar to that of carbonaceous chondrites. Pollack *et al.*[5] independently reached the same conclusion by comparing Phobos' spectral albedo with reflectance spectra of meteorites and basalts. Mass and density estimates (~2g cm^{-3}) for the satellite were made based on the perturbations on the orbit of Viking 1 by Phobos, during a series of close encounters[6]. The density of Phobos is consistent with the same composition as that inferred for its surface. Among materials expected to be common in the Solar System only ices or water-rich carbonaceous chondrites (20% H_2O by weight in type C1) have densities as low as Ceres and Phobos[7]. The compositional results have forced a re-evaluation of hypotheses concerning Phobos' origin. Scientific opinion now seems to favour an origin by capture of a body that came too close to Mars[8]. The analysis of data on Deimos reported here reveals a spectral reflectance very similar to that of Phobos, indicating a surface composition analogous to that of carbonaceous chondrites.

High-resolution pictures of Deimos obtained by Viking Orbiter 2 in recent close flybys showed a surface that appears to be morphologically different from that of Phobos[9]. Although the two satellites have equal densities of impact craters, on Deimos craters 50 m in diameter are filled in with fine-grained material, accounting for the relatively smooth appearance of the outer martian moon. Grooves are found over most of Phobos, but are conspicuously absent from the surface of Deimos. Why is the surface of Deimos but not Phobos mantled with large amounts of loose material, including clusters of house-sized blocks? One possibility is that the surfaces of the two satellites have significantly different mechanical properties. For example, an impact might produce a larger fraction of low-velocity fragments on Deimos than it would on Phobos. A second explanation is that it might be easier to knock material off Phobos than off Deimos, because Phobos is closer to Mars[10]. We will evaluate the first hypothesis by comparing the surface composition of the two satellites, that is by comparing the UV–visible reflectance spectrum of Deimos with that of Phobos.

The UV–visible reflectance spectrum of Deimos is compiled from the following sources: Mariner 9 UV spectrometry (UVS) and Canopus star tracker photometry, and ground-based colorimetry and polarimetry. Mariner 9 UV spectra of Deimos at a resolution of 1.5 nm were obtained on the 73rd revolution of the spacecraft around Mars. The phase angle of observation was 22°. Analysis of the Deimos spectra follows the procedure developed for Phobos[4] with the exception that a broader bandwidth was used, because the signal-to-noise ratio for Deimos was not as good as that for Phobos. Both preflight laboratory and inflight stellar calibrations show the absolute uncertainty of the UVS measurements to be ±20%, with a relative uncertainty of about ±7%. The relative uncertainty is shown in Fig. 1 as error bars.

Zellner and Capen[11] determined the slope of Deimos' polarisation curve at phase angles between 15° and 40°. Interpreting this slope on the basis of the new slope–albedo law[12,13], we arrive at a geometric albedo for Deimos in blue light of ≤ 0.057 (arrow in Fig. 1). They also made photoelectric observations of Deimos and derived a visual (V) geometric albedo of 0.070 ± 0.008, blue-visual colour index $B - V = 0.65 \pm 0.03$ and ultraviolet-blue colour $U - B = 0.18 \pm 0.03$, and phase coefficient $\beta_v = 0.036 \pm 0.05$ magnitude per degree for phase angles $< 20\%$.

Ground-based absolute photometry of the martian moons is very difficult due to their proximity to Mars (hence interference by light scattered from a bright primary). Photometric observations of Phobos from the martian surface with Viking Lander cameras did not have such a handicap[5]. We, therefore, made a new determination of Deimos' geometric albedo, using observations of the satellite made by the Mariner 9 Canopus star tracker (CST). Viewing Deimos from a Mars-orbiting platform avoids the problems caused by halation from Mars. Details of CST functions and data analysis are given elsewhere[14–17]. We shall first test CST data analysis on Phobos.

Following the procedure of Bowell and Zellner[18], we obtained Phobos' geometric albedo by extrapolating 83 CST measurements to a phase angle of 0°, using our own phase coefficient for angles 72–98° (ref. 19) and Noland and Veverka's β_v for 0–72° (ref. 20). [Similar values for the phase coefficients have been obtained by Klaasen *et al.*[21].] We assumed that Phobos' phase coefficient in the unobserved range of phase angles 0–18° is the same as that for the range 18–72°, because its phase curve is closely similar to that of Earth's moon (ref. 19), which has a nearly constant slope in the 0–70° region[22]. The average of 83 geometric albedo determinations is 0.046, with an absolute uncertainty of about ±0.01. It is slightly smaller than a similar broadband geometric albedo found from Viking lander imaging, 0.05±0.01 (ref. 5). The small difference is due to different assumptions made in analysing the Viking lander data. When extrapolating Viking lander observations, made at 35° phase, to 0° phase, we had assumed that Phobos' phase coefficient between 0° and 22° is the same as that of Deimos[4,5]. Because of the grossly different surface roughness of the two objects we now prefer a lunar-like phase curve between 0° and 72°. Using the same β_v as in the CST analysis we find that Viking Lander camera albedos for Phobos to be near 0.045 (see Fig. 1). The CST albedo for Phobos is plotted as a continuous line. The

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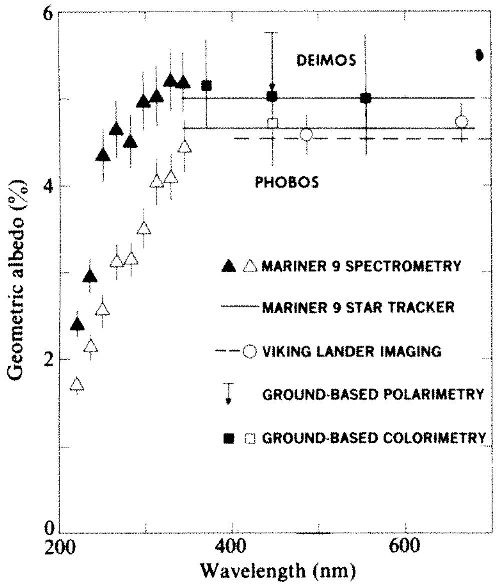

Fig. 1 The spectral albedo of the martian satellites. UV geometric albedos at 15-nm band centres: ▲, Deimos; △, Phobos. Photometry: ○, narrow band; – – –, —, broad band. Polarimetry ↓. Colorimetry: ■, Deimos; □, Phobos.

lengths of the lines indicate the bandwidths of the CST and Viking Lander camera broadband photometry. The CST has a blueish response so that CST magnitudes are similar to the commonly used photographic magnitudes. Kuiper's $B-V$ colour index for Phobos has been converted into a blue albedo after normalising to the Viking Lander imaging results[5] (open square in Fig. 1).

Following the same procedure as that for Phobos, analysis of 12 CST measurements yielded an average geometric albedo of 0.050, with an absolute uncertainty of about ±0.01 for Deimos. Zellner and Capen's colour indices were converted to *UBV* geometric albedos after normalising to the CST value. *B* and *V* albedos are the same for both Phobos and Deimos as the satellites have the same $B-V$ colour as the Sun. Viking Lander photometry has an absolute accuracy of ±20% and relative accuracy of ±5% (error bars). Ground-based data are accurate to ±0.01 (error bars). The ratio of Deimos' geometric albedo to Phobos' is now 1.1, as required by relative photometry of the satellites[20].

To a first approximation the reflectance spectra of Deimos and Phobos are very similar: both have low and flat reflectivities in the visible, dropping off sharply in the UV. Searches through spectral signatures of asteroids of known compositions and laboratory reflectance measurements of rocks, minerals and meteorites yielded carbonaceous chondrites as best matches for the spectral albedo of Phobos[3–5]. Only carbonaceous chondrites possess all three required spectral characteristics: low albedo, flat visible–IR reflectivity, with sharp downward bend in the UV. On the basis of a good match between the reflectance spectra of Deimos and Phobos, and with the composition of Phobos known to be similar to that of carbonaceous chondrites, it is reasonable to believe that the surface composition of Deimos is also similar to that of carbonaceous chondrites. Our identification of a carbonaceous chondrite composition for Deimos is consistent with the implications of a recent mass estimate for the satellite based on the perturbations on the orbit of Viking Orbiter 2 by Deimos, during a series of close encounters. This mass estimate yields preliminary densities that are equal to or less than the density of Phobos[23].

Why then do the surfaces of the martian satellites appear to be morphologically different? Any explanation requiring Phobos and Deimos to have surfaces with different mechanical strength seems to be unrealistic in view of their similar compositions and densities; instead, the states of the satellites' surfaces are probably due to their different orbital history.

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High time resolution riometer and X-ray measurements of conjugate electron precipitation from the magnetosphere

T. J. Rosenberg & J. C. Siren

Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742

L. J. Lanzerotti

Bell Laboratories, Murray Hill, New Jersey 07971

In this letter we describe the comparison of cosmic noise absorption data recorded by a riometer at Siple Station, Antarctica (76 °S; 84 °W; $L\sim4.2$) with simultaneous balloon measurements of bremsstrahlung X rays from the conjugate region near Roberval, Canada (48.3° N; 72.1° W). Both the cosmic noise absorption and the production of X rays are caused by the precipitation of energetic electrons ($E\geqslant15$ keV) from the magnetosphere. The time resolution and spatial coverage of the measurements enable us for the first time to show the similarity and simultaneity of rapid fluctuations of electron precipitation at ionospheric conjugate points. Furthermore, the data suggest that fast-response riometers are capable of resolving temporal structures in electron precipitation on a time scale which approaches that of electron microbursts (of the order of 1 s). The relative simplicity of riometers for the measurement of precipitating particle effects opens up important new avenues of research in studies of upper atmosphere ionisation phenoma.

The Siple riometer operates at 30 MHz and has a response time of 0.25 s to increases or decreases in signal intensity[1]. It utilises a zenith-directed dipole antenna with half-power at 30°. The antenna pattern projected to 100 km altitude covers a circular area of approximately 120 km diameter. The riometer data and the output of a three-axis fluxgate magnetometer are digitised at 2-s intervals and written in computer-compatible format on magnetic tape[2,3]. The X-ray measurements in the conjugate region were made at 35 km altitude with a zenith-directed uncollimated sodium iodide scintillation counter. An energy spectrum covering the range from 25 to 500 keV in seven

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differential channels is obtained with a time resolution of 0.01 s. The effective response of this detector projected to 100 km altitude covers a circular area equal to that of the Siple riometer. Calculations place the Siple conjugate point within 60 km of Roberval[4,5]. At the time of measurement, the balloon was located 30 km northeast of Roberval. Thus, portions of the effective areas of balloon and riometer gave overlapping coverage of the same field line(s). Uncertainty in the relative timing of the measurements in opposite hemispheres was ≤ 1 s.

A 1-min segment of X-ray, riometer and magnetometer data obtained during a microburst precipitation event on 9 July 1975 is presented in Fig. 1. Panel *a* illustrates the X-ray counting rate of the 45–65 keV energy channel at a resolution of 0.1 s. At this time, individual microbursts[6], the spikes of 0.2-s duration spaced 0.5–1 s apart, were occurring in groups separated by 5–10 s. The same X-ray data are plotted in Fig. 1*b* as 2-s averages, a reduction by a factor of 20 in time resolution. The Siple riometer and *D*-component magnetometer data, both limited in this event to the 2-s sampling rate, are shown in Fig. 1*c* and *d*, respectively.

It is evident on comparing *b* and *c* of Fig. 1 that each increase in X-ray counting rate at Roberval was followed several seconds later by an increase in cosmic noise absorption at Siple. Absorption variations as small as 0.02 dB are significant (the total ionospheric absorption caused by electron precipitation above Siple at this time was about 2 dB). At this resolution (2 s), the intensity–time profiles of absorption and X-ray pulses show some striking similarities. In general, however, the absorption pulses are somewhat broader than the associated X-ray pulses. This is to be expected from considerations of ionospheric response time even if the precipitation were identical at the conjugate points.

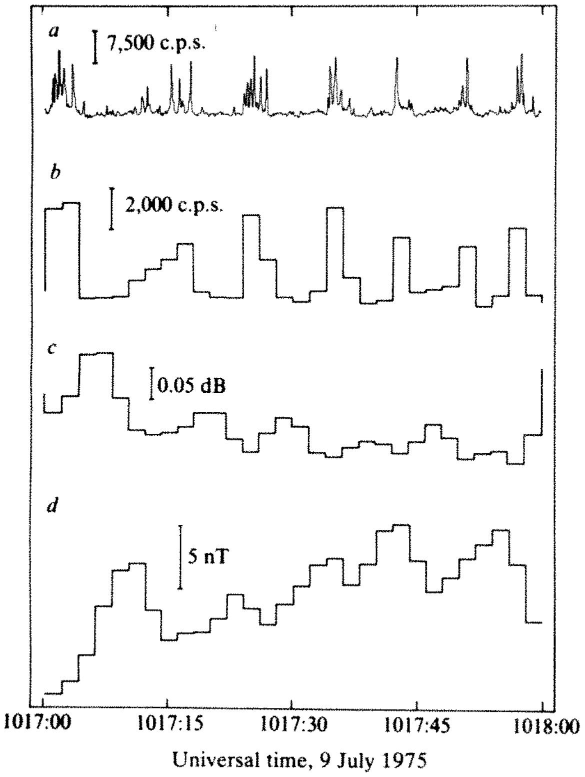

Fig. 1 *a*, Atmospheric bremsstrahlung X rays produced near Roberval, Canada ($L \approx 4.1$) by the precipitation of energetic electrons during a magnetic substorm. Individual microbursts are well resolved at 0.1-s resolution. *b*, The same X-ray data as in (*a*) but with a factor of 20 less resolution. The microburst structure is no longer apparent. *c*, Ionospheric absorption fluctuations at Siple Station, Antarctica (magnetically conjugate to Roberval) obtained by a 30-MHz riometer with resolution (2 s) equal to that of the X-ray data in *b*. Increasing absorption is upward. A time-delayed correlation (absorption lags X rays by ~4 s) is evident. *d*, Variations of the *D* component of the surface magnetic field at Siple at 2-s resolution. LT = UT − 5 hours.

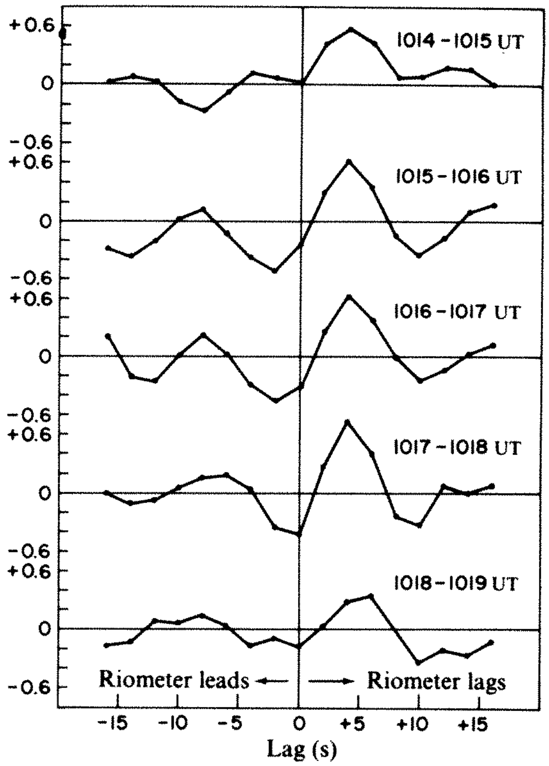

Fig. 2 Cross-correlation of 2-s resolution Siple riometer and Roberval X-ray data for consecutive 1-min segments. The correlation peak at +4 s exceeds the 99% confidence level in each of the first four segments.

Magnetic variations on a comparable time scale also occurred. Further details of the accompanying magnetospheric substorm and of the association of ultra low frequency (ULF) magnetic variations and absorption fluctuations have been given previously[2,7].

The data in Fig. 1 represent part of an extended period of disturbance. Figure 2 shows the results of cross-correlating the Roberval X-ray and Siple riometer data (2-s resolution) for consecutive 1-min segments between 1014 and 1019 UT. The peak at +4 s (riometer lags X rays) is confirmation that rapid fluctuations of electron precipitation are highly correlated at magnetic conjugate points. Approximately 1.8 s of the time delay is due to a phase shift introduced by a low-pass anti-aliasing filter in the riometer data system. For the data sets 1015–1016 and 1016–1017 UT, the probability of obtaining the observed correlation by chance is less than 0.001. For 1017–1018 UT the probability of obtaining the observed correlation by chance is less than 0.0001. For the interval 1015–1018 taken as a whole the probability is less than 1 in 10^8 that the correlation could have occurred by chance.

Previous studies of riometer pulsations accompanying X-ray[8] and luminosity[9] pulsations, using data from one end of a field line only, have found time delays of peak absorption of several seconds. This suggests, therefore, that the ≈ 2 s by which the absorption pulses lag the X-ray pulses in the present event may be accounted for by ionospheric response. Our investigation of this point will be presented elsewhere[10].

Because the data sampling rate did not take full advantage of the response capability of the Siple riometer, it was not possible to verify the presence of microburst structure in absorption. However, it should be possible to achieve finer time resolution. Considerations of ionospheric response suggest that absorption fluctuations caused by microbursts could be observed if the ionospheric time constant[8] [$\tau \approx (2\alpha N_e)^{-1}$, where N_e is the electron density and α is the effective recombination coefficient] were $\leqslant 5$ s, given the sensitivity (~ 0.02 dB) and instrumental time constant (~ 0.1 s) that can be achieved with riometers. Ionospheric time constants of this order may apply in the *D* region during absorption events associated with some magnetic storms and substorms.

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Charge dependence of pion production in heavy ion collisions

G. Bertsch

Department of Physics and Cyclotron Laboratory,
Michigan State University, East Lansing, Michigan 48824

Benenson *et al.*[1] measured the pion production cross-section in nuclear heavy ion collisions in the energy range 100–400 MeV per nucleon with the aim of determining whether collective effects associated with the larger number of nucleons were present in the pion field. An instability in the pion field, such as the putative pion condensation phase transition[2], would give rise to an enhanced number of physical pions. The expected production cross-section, in the absence of collective effects, is found from two simple models. One of these is a statistical model, in which the number of pions is calculated from a statistical equilibrium. At the other extreme, the nucleons in the target and projectile are assumed to be free and independent, except for their Fermi momentum, and create pions by two-body collisions. The experimental cross-section at the lowest energy was found to be larger than either model by at least an order of magnitude[3], suggesting that collective effects are indeed present. Interestingly, the cross-section had unexpectedly strong dependence on the charge of the pion produced. At the highest energy studied, 400 MeV per nucleon, many more π^- than π^+ were produced in the forward direction. At the lowest energy studied, 100 MeV per nucleon, the relative yields are reversed, with more π^+ produced. The experimental ratios of π^- to π^+,

$$R_{-/+} = \frac{\mathrm{d}\sigma_{\pi^-}}{\mathrm{d}^3p} \Big/ \frac{\mathrm{d}\sigma_{\pi^+}}{\mathrm{d}^3p}$$

are quoted in Table 1. Benenson *et al.*[1] explain the π^- enhancement in terms of the Coulomb distortions of the pion wavefunctions. Here I include in the same framework an explanation of the low energy result.

Table 1 Experimental ratios of π^- to π^+

E_{proj} (MeV per nucleon)	Pion kinetic energy (MeV)	$y_\pi - y_{\text{proj}}$*	$R_{-/+}$
~100	34	0.23	0.7
	54	0.40	0.6
	76	0.54	0.5
400	34	−0.20	3.0
	54	−0.04	10.0
	76	0.11	2.5
	123	0.36	1.4
	155	0.49	1.2

* Rapidity, the Lorentz-additive measure of velocity, is defined as $y = \frac{1}{2}\ln(E+pc)/(E-pc)$.

My starting point for the pion production rate is the formula

$$W = 2\pi \sum_{\mathrm{f}} \langle \mathrm{i}|H_{\pi\mathrm{N}}|\mathrm{f}\rangle^2 \rho_{\mathrm{f}}$$

where ρ_{f} is the density of final states and $H_{\pi\mathrm{N}}$ is the pion creation part of the nuclear hamiltonian. The ρ_{f} is a product of density of pionic states ρ_{f}^{π}, and density of nucleonic states $\rho_{\mathrm{f}}^{\mathrm{N}}$. Normalising the pion wavefunction to plane waves at infinity, the pionic density of states is the usual $\rho_{\mathrm{f}}^{\pi} = \frac{\mathrm{d}^3p}{(2\pi)^3}$. The transition matrix element $\langle \mathrm{i}|H_{\pi\mathrm{N}}|\mathrm{f}\rangle^2$ will be proportional to the probability of these normalised wavefunctions in the collision region, $|\phi_{\pi^\pm}|^2$. Benenson *et al.*[1] compute the charge dependence as

$$R_{-/+} \approx \frac{|\phi_{\pi^-}(R)|^2}{|\phi_{\pi^+}(R)|^2}$$

where $\phi_{\pi^\pm}$ contains the Coulomb distortion of the projectile. Thereby the large effect seen for pion velocities close to the beam velocity is qualitatively explained. The effect of the nuclear density of states will now be included. To describe $\rho_{\mathrm{f}}^{\mathrm{N}}$, it is assumed that there is a thermal equilibrium among nucleons. The final state density then depends on pion energy and charge according to

$$\rho_{\mathrm{f}}^{\mathrm{N}} \sim \exp\left[-(E_{\pi^\pm} \mp (\mu_{\mathrm{p}} - \mu_{\mathrm{n}}))/kT\right] \qquad (1)$$

Here kT is the temperature of the nucleons, and μ_{p} and μ_{n} are the chemical potentials of the protons and neutrons. In view of the relatively small number of nucleons involved in the collision, and the fact that the pions are most likely to be created at the initial stages of the collision, before any sort of equilibrium is established, the statistical assumption may be questioned. However, it is a fact that energy spectra tend to be exponential, with a coefficient close to the 'temperature' calculated as below[4]. Thus we at least find phenomenological justification for equation (1).

$R_{-/+}$ is now compared at the two energies, for the same velocity of the pion with respect to the projectile. The Coulomb distortion effects should be nearly the same, so the ratio of $R_{-/+}$ would be given by

$$R_{-/+}/R'_{-/+} \approx \exp\left[2(\mu_{\mathrm{p}} - \mu_{\mathrm{n}})\left(\frac{1}{kT'} - \frac{1}{kT}\right)\right]$$

In the system studied experimentally, $^{20}\mathrm{Ne} + \mathrm{NaF}$, the chemical potentials differ mainly because of the Coulomb field. Assuming that the pion production takes place in an overlap region between the colliding nuclei, the difference between chemical potentials may be estimated as

$$\mu_{\mathrm{p}} - \mu_{\mathrm{n}} = \frac{Ze^2}{R} \approx \frac{20(1.44)}{3.3}\,\mathrm{MeV} \approx 8.7\,\mathrm{MeV}$$

To estimate the temperature at 100 MeV per nucleon projectile energy, note that the energy available in the centre of mass, per nucleon, is ~25 MeV. With Boltzmann statistics, this implies a temperature of $\frac{2}{3}(25) = 16.7$ MeV. A more detailed calculation taking into account such things as quantum statistics and binding energy effects yields a temperature of 13.5 MeV. Similar considerations for 400 MeV per nucleon yield a temperature of

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55 MeV. Then for a given pion velocity with respect to the projectile, we expect a diminution of π^- at the lower energy of

$$R/R' \approx \exp\left(17.4\left(\frac{1}{55}-\frac{1}{13.5}\right)\right) \approx 0.37$$

This agrees with the experimental ratio $0.5/1.2 \approx 0.42$ found for pions of high rapidity.

From these considerations, it seems that $R_{-/+}$ will be a useful probe of the nucleonic density of states in the early stages of heavy ion collisions at lower bombarding energies, and seems to be conveniently interpretable as a temperature. This complements the usefulness of $R_{-/+}$ at higher energies as a probe of the geometry of the collisions, via the dependence on the Coulomb distortions.

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Improved planar solar convertor based on uranyl neodymium and holmium glasses

Renata Reisfeld & Yehoshua Kalisky

Department of Inorganic Chemistry and Energy Center,
The Hebrew University, Jerusalem, Israel

The use of uranyl doped glasses for solar energy conversion and concentration was discussed by Reisfeld and Neuman[1]. While the UO_2^{2+} ion is an efficient energy convertor, its maximum emission peaking around 500 nm does not coincide with the maximum sensitivity of the existing solar cells which is around 700–1,000 nm. The use of Nd^{3+} doped glasses for solar energy collectors was described by Levitt and Weber[2]. While the emission of Nd^{3+} peaking at 880 nm ($^4F_{3/2}\rightarrow{}^4I_{9/2}$) and at 1.06 μm ($^4F_{3/2}\rightarrow{}^4I_{11/2}$) is ideal for matching with the maximum sensitivity of solar cells, its absorbance arising from the forbidden transition 4f–4f is low and the overlap of the absorption spectrum of Nd^{3+} with the solar spectrum is rather weak. To increase the operational efficiency of solar collectors, a maximum overlap between the absorption spectrum of the collector and the solar spectrum is required[3]. In this report we suggest that glasses doped by uranyl and neodymium and uranyl and holmium increase the response to the solar spectrum, thus increasing the collection efficiency, and convert the absorbed light in the wide spectral range into the narrow fluorescence band at 1.06 μm and 880 nm in uranyl–neodymium glasses and 660 nm ($^5F_5\rightarrow{}^5I_8$) and 750 nm ($^5F_4, {}^5S_2\rightarrow{}^5I_7$) in the uranyl–holmium co-doped glasses.

Energy transfer from $UO_2^{2+}\rightarrow Nd^{3+}$ in barium crown glasses was discussed by Gandy *et al.*[4] and Melamed *et al.*[5] for small concentrations of UO_2^{2+} in the glasses. The use of energy transfer for lowering the laser threshold in rare earth doped glasses is a well-known phenomenon[6]. Here we present quantitative calculations on energy transfer between $UO_2^{2+}\rightarrow Nd^{3+}$ and $UO_2^{2+}\rightarrow Ho^{3+}$ at a much higher concentration range than in ref. 4. This higher concentration range is suitable for solar energy conversion. The absorption, excitation and emission spectrum of UO_2^{2+} doped phosphate glasses may be found in ref. 1.

The excitation spectrum of the $^4F_{3/2}\rightarrow{}^4I_{11/2}$ (1.06 μm) transition of a glass doped by 2 wt % Nd^{3+} and co-doped by 2 wt % Nd^{3+} + 1 wt % UO_2^{2+} in the spectral range 360–500 nm is presented in Fig. 1. Due to the energy transfer from UO_2^{2+} to Nd^{3+}, the integrated fluorescence intensity of Nd^{3+} excited in this region is increased 8.5-fold. In the total spectral range from 360–800 nm this increase amounts to 150%. This percentage is equal to the number of photons of the solar spectrum affecting the co-doped glass divided by the number of photons affecting the Nd^{3+}-only doped glass. This ratio was calculated using the formula:

$$\frac{S_{cod}}{S_{dop}} = \frac{A^1\times\eta_{tr}\times\eta^1 + A^2\times\eta^2}{A^2\times\eta^2}\times 100 \tag{1}$$

Where S_{cod} is area under the excitation spectrum of the co-doped glass, and S_{dop} the area under the excitation spectrum of the Nd^{3+} doped glass. The area of the excitation spectrum was corrected for the spectral response of the xenon source and the excitation monochromator. η_{tr} is energy transfer efficiency from UO_2^{2+} to Nd^{3+}, A^1, A^2 = absorbance of UO_2^{2+} and Nd^{3+} respectively, and η^1, η^2 = fluorescence quantum efficiency of UO_2^{2+} and Nd^{3+} respectively.

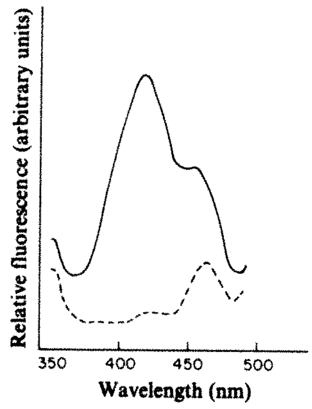

Fig. 1 Excitation of Nd^{3+} (– – –) and $Nd^{3+} + UO_2^{2+}$ (——). Emission monitored at 1,055 nm.

The emission spectrum of Nd^{3+} and Ho^{3+} glasses co-doped with UO_2^{2+} are presented in Fig. 2. The emission maxima correspond to the maximum sensitivity of the silicon solar cells[7]. The efficiency of energy transfer was calculated using equations (2) and (3) and is presented in Table 1.

$$\eta_{tr} = 1 - \frac{\tau_d}{\tau_d^0} \tag{2}$$

$$\eta_{tr} = 1 - \frac{I_d}{I_d^0} \tag{3}$$

Where τ_d is the first *e*-folding time[6] of UO_2^{2+} in the presence of Nd^{3+} and τ_d^0 is the first *e*-folding time of UO_2^{2+} alone. I_d and I_d^0

Fig. 2 Fluorescence emission spectra of Nd^{3+} and Ho^{3+} co-doped with UO_2^{2+} when excited into the maximum absorption peak of UO_2^{2+}.

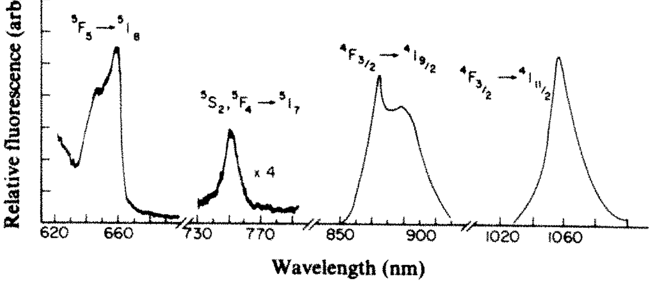

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Table 1 Energy transfer efficiency between $UO_2^{2+} \rightarrow Nd^{3+}$ and $UO_2^{2+} \rightarrow Ho^{3+}$ using equations (2) and (3) (excitation at 420 nm)

Concentration (wt %)	Decay time (μs)	η_{tr} eqn (2)	η_{tr} eqn (3)
1 wt % UO_2^{2+}	205		
1 wt % UO_2^{2+} + 2 wt % Nd^{3+}	90	0.56	0.65
1 wt % UO_2^{2+} + 2 wt % Ho^{3+}*	145	0.29	0.55

* The discrepancy of η_{tr} obtained by the two methods in the case of Ho^{3+} will be discussed in a following paper.

are the fluorescence intensities of UO_2^{2+} with Nd^{3+} and UO_2^{2+} alone respectively.

From the above it can be seen that the efficiency of the previously proposed fluorescent collector can be significantly increased by utilisation of energy transfer from UO_2^{2+} to Nd^{3+}.

The practical application of Ho^{3+} co-doped glass is still doubtful because of the low branching ratio of the $^5F_4$, $^5S_2 \rightarrow {}^5I_2$ and $^5F_5 \rightarrow {}^5I_8$ transitions.

We thank Enrique Berman and Professor Fred Singer for their interest in this work. Patents for the convertor described here are pending.

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Zirconolite—a fluorite-related superstructure

H. J. Rossell

Engineering Ceramics and Refractories Laboratory, CSIRO Division of Materials Science, PO Box 4331, GPO, Melbourne 3001, Australia

Synthetic zirconolite, $CaZrTi_2O_7$, has attracted interest recently, as it has been proposed as a constituent phase of an artificial rock (SYNROC) which may immobilise, in solid solution, the elements occurring in high-level nuclear reactor wastes[1]. Although it is known that zirconolite is a superstructure phase, derived by ordering of an anion-deficient fluorite (CaF_{2-x} or Mo_{2-x}) type of substructure[2], the crystal structure proper, and thus the nature of the ordering, is not known. This has hampered discussion of the crystal chemistry of the phase. A crystal structure determined from powder X-ray diffraction intensities and adequate for such discussion purposes is reported here.

Single-phase $CaZrTi_2O_7$ was made by heating the component oxides mixed in stoichiometric proportions at 1,450 °C for 4 days. Some of the product was crushed and examined in an electron microscope fitted with a tilting stage. Single crystal fragments were selected, and appropriate electron diffraction patterns were recorded. These confirmed the published crystallographic relationship of the supercell to the subcell[2], and enabled the space group of the supercell to be identified as either the centrosymmetric C2/c or the non-centrosymmetric Cc. Lattice parameters were determined from a powder X-ray diffraction photograph taken with a Guinier camera using CuKα_1 radiation and with thoria ($a = 5.5972$ Å) as an internal standard (Table 1).

Well crystallised zirconolite may be prepared quite readily[2,3], but at least one attempt to determine the structure from single-crystal X-ray diffraction data has been unsuccessful (W. G. Mumme, personal communication). It was suspected that the data may have been affected by multiple twinning in the crystal: this frequently accompanies the crystallisation of materials with a high degree of pseudosymmetry, and may not be readily detectable. Therefore, powder X-ray diffraction intensity data have been used to determine the structure. This technique has been successful for some other oxides with fluorite-related superstructures which could not be prepared as suitable single crystals.

Powder X-ray diffraction intensities for $\theta < 40°$ were recorded on chart, using a powder diffractometer and filtered CuKα radiation. Peaks were separated where practicable, and their areas measured with a planimeter. A reflection or group of reflections was assigned to each such observation, giving a data set composed of 295 reflections distributed among 155 observations. Structure refinement was carried out using a full matrix least-squares computer program for the treatment of powder data.

As the unit cell is composed of eight fluorite M_4O_8 subcells, it has the contents $Ca_8Zr_8Ti_{16}O_{56}\square_8$ (□, oxygen vacancy), an assignment confirmed by density measurement. The structure was determined by trial and error according to procedures used in similar circumstances[4–6]. Trial structures, comprising all possible ordered arrangements of metal atoms allowed by the space groups, with atoms on the ideal fluorite positions of the supercell and all oxygen sites occupied, were refined, due care being taken in the removal of initial degeneracy. These trial structures were rejected or retained according to the value of the crystallographic residual R, which expresses the agreement between observed and calculated intensities.

It was found that one structure in C2/c (and its equivalent in Cc) was greatly superior to all the others. With this model, the site of the oxygen vacancy was found by refining the occupancies of the eight oxygen atoms in the asymmetric unit, when one fell immediately to zero, while the others were unaffected. The extent of disorder in the metal atom array was assessed by refining cation occupancies. The refinement converged satisfactorily to $R = 0.054$, with reasonable values for the refined parameters and their calculated standard deviations. An overall isotropic temperature factor was used, and on refinement, it assumed a value close to zero, with a relatively large standard deviation. As powder work involves low-angle data and non-ideal specimen surfaces, the temperature factor may have little significance as a structural parameter.

There was no indication that the structure was not centrosymmetric: refinement of the trial structure in Cc (which has 4-fold sites only) produced the same structure as that found in C2/c within calculated standard deviations of atomic parameters, and there was no significant improvement in the fit of observed and calculated intensities, despite the greater number of variable parameters. Also, a test for the absence of a

Table 1 Crystallographic data for zirconolite, $CaZrTi_2O_7$

Unit cell:
$a = 12.4458(7)$* Å, $b = 7.2734(4)$ Å,
$c = 11.3942(9)$ Å, $\beta = 100.533(7)°$.
Space group: C2/c (No. 15, C_{2h}^6) (ref. 11).
$Z = 8$. $D_x = 4.442$ g cm^{-3} $D_m = 4.45(5)$ g cm^{-3}
Relationship of supercell and subcell axes:

$$\begin{pmatrix} a \\ b \\ c \end{pmatrix} = \begin{pmatrix} -1 & -1 & 2 \\ 1 & -1 & 0 \\ 3/2 & 3/2 & 1 \end{pmatrix} \begin{pmatrix} a_1 \\ a_2 \\ a_3 \end{pmatrix}$$

* Numbers in parentheses are calculated standard deviations, which apply to the last quoted place.

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Table 2 Structure parameters

Atom	Point set	Occupancy	x	y	z
M1	8(f)	Ca	0.3718(12)*	0.1245(19)	0.4952(9)
M2	8(f)	0.93(3)Zr +0.07Ti†	0.1225(6)	0.1222(10)	−0.0258(5)
M3	8(f)	Ti	0.2498(11)	0.1223(21)	0.7465(9)
M4	4(e)	0.86Ti +0.14Zr	$\frac{1}{2}$	0.055(2)	$\frac{1}{4}$
M5	4(e)	Ti	0	0.127(3)	$\frac{1}{4}$
X1	8(f)	0	0.310(3)	0.133(6)	0.275(3)
X2	8(f)	0	0.470(3)	0.146(5)	0.102(3)
X3	8(f)	0	0.197(4)	0.083(5)	0.573(3)
X4	8(f)	0	0.403(3)	0.174(5)	0.719(3)
X5	8(f)	0	0.702(4)	0.169(4)	0.590(3)
X6	8(f)	0	−0.001(3)	0.111(5)	0.414(3)
X7	8(f)	0	0.119(4)	0.055(4)	0.788(3)
X8	8(f)	□	$\frac{5}{32}$	$\frac{1}{8}$	$\frac{3}{16}$

Mean distances in Å

Ti–O	1.95(7)	Ti–□	2.21(2)	M–O (fluorite)	2.175
Zr–O	2.19(14)	Zr–□	2.396(5)		
Ca–O	2.49(7)				
O–O	2.76(14)	O–□	2.01(9)	O–O (fluorite)	2.512

* Numbers in parentheses are calculated standard deviations, which apply to the last quoted place.

† There is only one formal occupancy variable, because of the stoichiometry restraint.

centre of symmetry, based on the detection of nonlinear optical effects[7], yielded a null result.

The structural parameters determined are collected in Table 2. In this structure, Ca atoms are coordinated by eight oxygen atoms at the corners of a distorted cube, Zr atoms (overlooking the minor components at mixed-occupany sites for convenience) by oxygen at seven of the eight vertices of a distorted cube, and Ti by six oxygen atoms, either at the vertices of octahedra (M3 and M5) or a trigonal prism (M4). All these results accord with crystal-chemical expectations[8,9]. The 'vacant' oxygen sites occur in pairs, separated by a lattice vector of the type $\frac{1}{2}$ [111] (fluorite), with a Ti atom M3 or M5 in between, and, as expected on electrostatic grounds, the relaxation of atoms from their ideal fluorite positions is such that the tetrahedron of metals (cations) surrounding a vacancy is expanded, whereas the octahedron of oxygens around it is contracted. Similar effects have been found in all of the anion-deficient fluorite-related superstructures studied so far.

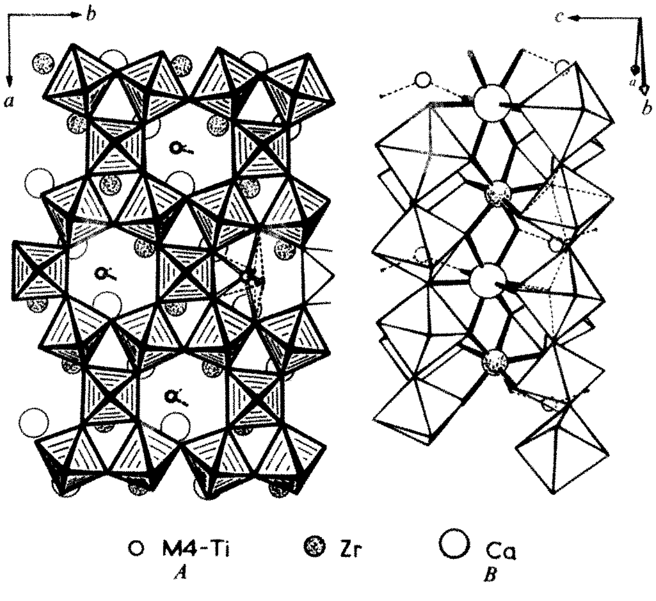

Fig. 1 A, (001) sheet of TiO_6 octahedra with parallel plane of Ca + Zr below it. The Ti atoms M4 in the sheet are associated with two O atoms X2, which are not in the octahedra: The trigonal prismatic coordination of one M4 is outlined. B, Portion of the structure viewed down [1$\bar{1}$0] approximately, showing coordination of Ca and Zr.

In the superstructure, there are planes of metal atoms parallel to (001) which are derived from the f.c.c. fluorite 'close-packed' metal-only (111) planes. The cation ordering is such that these planes alternately contain Ti only (A planes) and Ca + Zr (B planes). The (001) sheets of TiO_6 octahedra (Fig. 1) are similar to the sheets of octahedra parallel to {110} in the pyrochlore structure[10]. Within each B layer, Ca and Zr occupy alternate rows, which run parallel to [110] and [1$\bar{1}$0] in alternate B planes.

This structure determination provides an adequate basis for discussion of the crystal chemistry of zirconolite. However, crystals suitable for X-ray study have been produced in the CSIRO Division of Mineral Chemistry, and the structure has been determined independently from single crystal data, using direct methods. The refined results are more precise than those above, and reveal some interesting new structural effects; nonetheless, the overall agreement between the two determinations is excellent. The single crystal work has been done jointly with I. E. Grey, R. J. Hill and A. F. Reid (Mineral Chemistry) and B. M. Gatehouse (Monash University), and will be published elsewhere. Also, a structure study of non-stoichiometric zirconolite, and of zirconolite containing various oxides in solid solution has been completed and will be reported in the near future.

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OCS, stratospheric aerosols and climate

R. P. Turco*, R. C. Whitten†, O. B. Toon†, J. B. Pollack† & P. Hamill‡

* R & D Associates, PO Box 9695, Marina del Rey, California 90291
† NASA Ames Research Center, Moffett Field, California 94035
‡ Systems and Applied Sciences Corporation, Hampton, Virginia 23665

Carbonyl sulphide (OCS) is found to be the predominant sulphur-bearing compound in our atmosphere[1–3]. It contributes to the formation of stratospheric sulphate aerosol particles[4], which affect the Earth's radiation balance and climate[5–7]. Using recently obtained data, we estimate that OCS has a global source of ~5 tg per year (tg = 10^{12} g) and a lifetime of roughly 1 yr. We calculate that increasing anthropogenic emissions of OCS could cause measurable climate alterations within the next century.

Numerous sources of OCS have been identified (see Fig. 1). Crutzen *et al.*[8] estimate that natural and agricultural fires contribute 0.2–0.3 tg of OCS to the atmosphere each year. Adams *et al.*[9] measured average OCS emission rates for a variety of common soils of about 0.004 g m^{-2} yr^{-1}, which may be extrapolated to a global OCS source of nearly 0.5 tg yr^{-1}. Adams *et al.*[9] also noted OCS emissions several thousand times greater than average above saline marshes. Carbonyl sulphide has been detected near cattle feedlots in concentrations as high as 6,000 p.p.b.v.[10]. Volcanoes and fumaroles seem to represent a minor source of OCS (refs 11, 12). We estimate that the direct contributions of biospheric processes to the OCS budget may be ~1 tg yr^{-1}.

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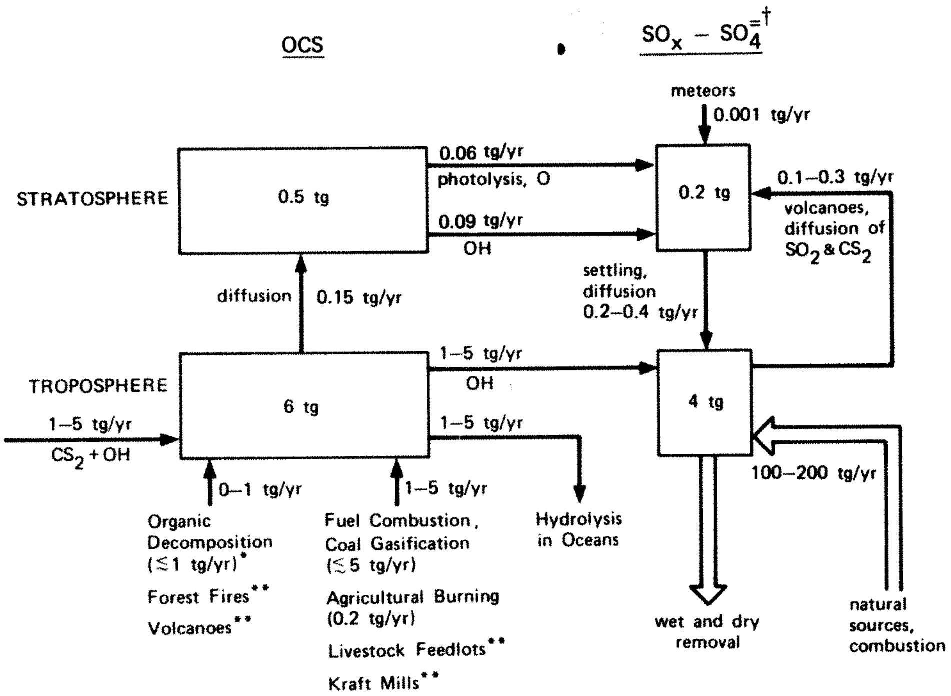

Fig. 1 Atmospheric budget of carbonyl sulphide, and of other sulphur compounds. Masses are given in teragrams (tg). (The small mass difference betwween OCS and SO_2 molecules is ignored.) † Masses are expressed in terms of an equivalent amount of SO_2. * The source is difficult to quantify, but appears to be significant. ** The source is small, probably much less than 1 tg yr^{-1}.

Kurylo[13] has suggested that carbon disulphide is a precursor of OCS via the reaction

$$CS_2 + OH \rightarrow OCS + SH (k = 1.9 \times 10^{-13}\ cm^3\ s^{-1} \text{ at } 296\ K) \quad (1)$$

Using Kurylo's rate constant and observed daytime OH concentrations[14–17], the CS_2 lifetime against conversion into OCS appears to be ~1–3 months[18]. CS_2 concentrations of 0.07–0.37 p.p.b.v. have been measured by Sandalls and Penkett[19] in the UK. Interestingly, these amounts are roughly consistent with the quantity of CS_2 found dissolved in the Atlantic Ocean by Lovelock[20] (~5×10^{-13} g ml^{-1}) assuming equilibrium between the atmosphere and oceans. Adopting a uniform tropospheric CS_2 concentration of 0.07 p.p.b.v. (taking the lowest value observed by Sandalls and Penkett as the background abundance), an average CS_2 lifetime of two months, and unit conversion of CS_2 into OCS, we estimate that 5 tg yr^{-1} of OCS could be generated from CS_2.

Sources of atmospheric CS_2 could include the oceans[20], marshes[9] and industrial processes[21,22]. Until more quantitative information concerning the global budget of CS_2 is gathered, however, we favour an estimate of 1–5 tg yr^{-1} of OCS from the decomposition of natural and manmade CS_2.

The refining and combustion of fossil fuels currently releases about 100–150 tg of SO_2 to the atmosphere each year. The average sulphur residue in coal is ~1% by mass, and in petroleum, 0.2% (refs 25, 26). Assuming that 6% of the emitted sulphur is OCS (where 6% is a maximum value for existing sulphur recovery systems[21]) the OCS combustion source could be 6–9 tg yr^{-1}. Of course, only a small fraction of worldwide combustion emissions are processed for sulphur removal, and untreated stack gases generally have very low OCS concentrations[21]. Hence, the OCS emission might be less than 1 tg yr^{-1}.

During coal gasification and liquification, highly reducing conditions prevail, and OCS may be produced in fractional quantities as large as 10^{-3} of the total gas volume. After scrubbing of the tail gases, however, emissions of OCS from these coal plants should (typically) amount to less than 1% of the sulphur in the coal processed[21]. Catalytic converters used in cars occasionally generate large quantities of H_2S and OCS (ref. 27). Although car petrols have very low sulphur contents—commonly 0.03–0.06% S by weight (ref. 21)—all of the sulphur is released to the atmosphere. Thus, a small contribution to the global OCS budget from petrol consumption could be expected. Negligible anthropogenic sources of OCS include direct commercial production[21], cigarette smoke[28], vapours from cooking grain mashes[29], and Kraft mills[30].

We conclude that the total global source of atmospheric OCS may be in the range of 1 to 10 tg yr^{-1} (see Fig. 1), and that a large fraction of this, perhaps as much as 50%, may result from anthropogenic activities related mainly to fuel processing and consumption.

Ultraviolet photolysis of OCS above 20 km is a sink for OCS and a source of sulphur for stratospheric aerosols[4], but leads to an overall OCS atmospheric lifetime of 50 years or more. Reaction with OH may be the largest atmospheric

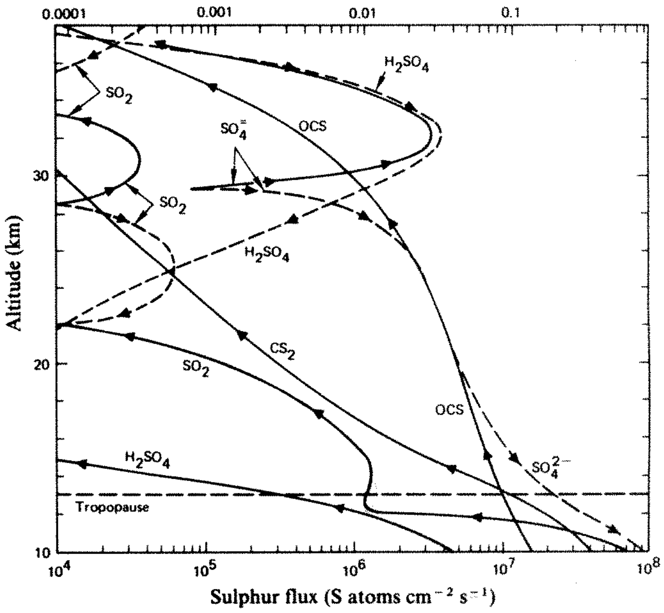

Fig. 2 The stratospheric balance of gaseous sulphur and aerosol sulphate in a one-dimensional model[33,34]. Equivalent sulphur atom fluxes are given. The arrows indicate direction of sulphur flow (also, solid lines correspond to upward fluxes, dashed lines to downward fluxes). The SO_2 flux divergence is complicated because of SO_2 sources near 15 km due to CS_2 decomposition, 29 km due to OCS photodissociation, and 50 km due to H_2SO_4 photolysis[33]. An H_2SO_4 source exists above 30 km as a result of aerosol droplet transport and evaporation[34].

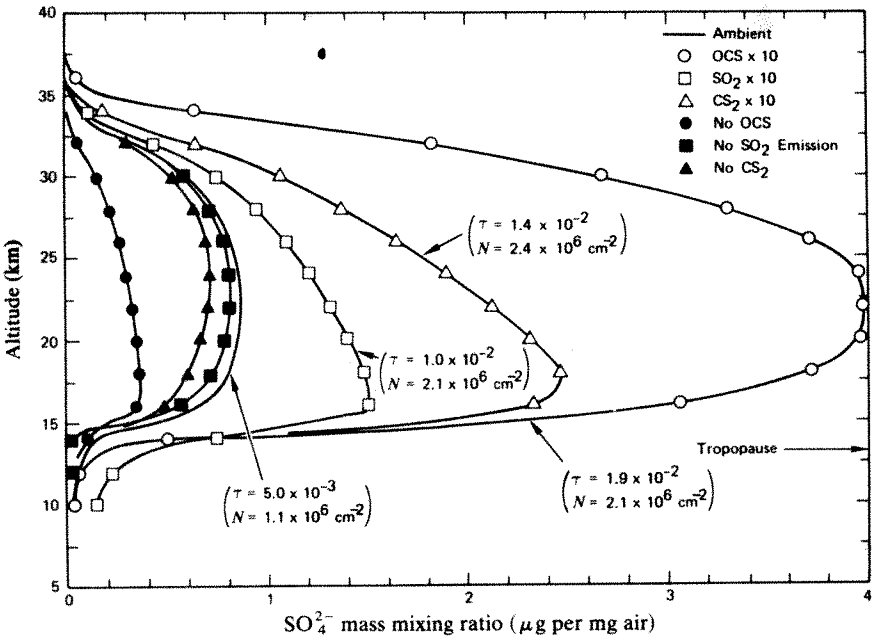

Fig. 3 Stratospheric aerosol sulphate (SO_4^{2-}) mass mixing ratios predicted for ambient and perturbed conditions. A 10-fold enhancement in OCS, SO_2 or CS_2 is simulated by increasing its surface concentration 10-fold, resulting in roughly a factor of 10 increase in its surface emission rate and tropopause concentration, while the abundances of the other two constituents are essentially unaltered. For several of the cases treated, the total large particle ($r > 0.15$ μm) column concentration in the stratosphere (N) and the optical depth of the sulphate layer at 550 nm (τ) are given in brackets.

sink for OCS[13],

$$OCS + OH \rightarrow CO_2 + SH (k = 5.7 \times 10^{-14} \text{ cm}^3 \text{ s}^{-1} \text{ at } 296 \text{ K}) \quad (2)$$

For likely tropospheric OH concentrations[14–17] reaction (2) implies roughly a one-year atmospheric lifetime for OCS. Reactions of OCS with ground state and excited oxygen atoms can be ignored.

Although hydrolysis of OCS is rapid in basic solutions, it is quite slow (weeks to months) in neutral[21] and acidic[31] solutions. OCS also has only a moderate solubility (1.4 g l^{-1} H_2O STP). Accordingly, we deduce that scavenging of OCS by clouds, aerosols and rainfall may be neglected. Seawater, by contrast, has a *p*H greater than 7, which implies efficient OCS hydrolytic decomposition. It has been estimated (F. S. Rowland; personal communication) that oceanic hydrolysis could lead to a global OCS lifetime as short as a year.

Carbonyl sulphide has been measured extensively in the troposphere[1–3,19] and lower stratosphere[32] and is the predominant sulphur-bearing compound in the atmosphere. OCS has a ubiquitous tropospheric concentration of about 0.5 p.p.b.v. with no apparent gradient between the boundary layer and the upper troposphere. OCS also has a small (~5%) interhemispheric contrast[3]. In addition, OCS variability (from one measurement to another) is found to be less than 10% (refs 2, 3, 19). All these observations are consistent with an OCS lifetime of the order of one year.

With reference to Fig. 1, the 6 tg of OCS measured in the atmosphere is compatible with an OCS source of 6 tg yr^{-1} and a tropospheric lifetime of one year, similar to the values we have estimated. Recent model calculations of the ambient atmospheric sulphur balance[33,34] indicate that observed OCS, CS_2 and SO_2 abundances are roughly consonant with global surface emission rates of 3 tg yr^{-1} OCS, 4 tg yr^{-1} CS_2 and 100–150 tg yr^{-1} SO_2.

The stratospheric sulphur balance for non-volcanic conditions is shown in Fig. 2 (based on a one-dimensional model calculation[33]). The dominance of OCS is clearly brought out, although CS_2 and SO_2 also contribute significantly to the stratospheric sulphur cycle. This raises the possibility that increased fossil fuel consumption rates, as have been projected by Rotty[35] (approximately a fourfold increase by 2025, to 23,000 tg carbon per year), may cause greater sulphur vapour emissions, which can alter the stratospheric sulphate aerosol, or Junge[36], layer. Using an appropriate model[33,34], we have studied the sensitivity of aerosol mass loading to changes in OCS, SO_2 and CS_2 concentrations (Fig. 3). Obviously, changes in the OCS level have the largest impact on the aerosol layer. Even so, a 10-fold increase in CS_2 emissions could lead to a greater aerosol enhancement. Most of the CS_2 emissions might be chemically converted into OCS and SO_2 in the lower atmosphere. Hence, if CS_2 is the primary source of OCS, a CS_2 increase could produce an effect nearly as large as the combined effects of the CS_2 and OCS increases shown in Fig. 3.

The ambient aerosol layer has an optical depth of about 0.005 at 550 nm (ref. 37). For a 10-fold increase in OCS, the estimated optical depth is about 0.019. This can be compared to the optical depths observed following volcanoes Fuego (0.02–0.03) and Agung (0.02–0.04 in the Northern Hemisphere, and 0.2–0.3 in the Southern Hemisphere). Thus, a 10-fold increase in OCS produces an optical depth perturbation comparable to that of a modest volcanic eruption.

When we use these OCS optical depths in an improved version of the radiation transport model of Pollack *et al.*[5–7], we find a possible decrease in the average global surface temperature of about 0.1 K, and a possible comparable increase in stratospheric temperatures. The surface temperature change is at the threshold of climatic significance. However, OCS, like CO_2, can create a 'greenhouse' effect in which far IR radiation emitted by the Earth is trapped in the lower atmosphere, warming its surface and compensating somewhat the aerosol cooling.

Because of man's apparently strong influence on the atmospheric sulphur cycle, and because of the established role of sulphur emissions in air pollution episodes and possible climatic changes, we suggest that future use of fossil fuels be carefully monitored for released sulphur compounds, including OCS and CS_2.

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Low-aspect ratio ignimbrites

G. P. L. Walker, R. F. Heming & C. J. N. Wilson*

Department of Geology, University of Auckland, Auckland,
New Zealand

Conventionally[1], three important characteristics of ignimbrites are that they show a very pronounced tendency to pond in topographic depressions, possess a flat, horizontal or gently sloping upper depositional surface, and have a thickness generally between 10 and 1,000 m. The youngest major ignimbrite, that of 1912 in the Valley of Ten Thousand Smokes (Alaska)[2–4] shows these characteristics well, being ponded in a valley 25 km long with a flat upper surface sloping down-valley at an average of 1.3°, and having an estimated thickness exceeding 100 m in places. All three characteristics are commonly used as field criteria to distinguish ignimbrites from other pyroclastic rock bodies. Here we look at certain ignimbrites which depart significantly from this conventional form in that a major part of them occurs as a thin layer mantling the landscape, resting on slopes as steep as 30°, and with an upper surface sensibly parallel with the underlying surface. We have studied two examples, the 1,800-yr-old[5] Taupo ignimbrite (New Zealand), and the 1,400-yr-old Rabual ignimbrite (New Britain)[6,7]. We also cite several others. These ignimbrites have a remarkably low aspect ratio. This ratio, previously applied to lava extrusions[8,9], provides a useful means of quantifying the overall geometry of rock bodies. The ratio is that of average thickness to lateral spread; conveniently the latter is taken as the diameter of the circle which covers the same area as the rock body.

The young age of the Taupo and Rabaul ignimbrites, and their occurrence in humid areas where a luxuriant vegetation anchors and protects pyroclastic deposits very soon after they have formed, has resulted in their remarkably good structural preservation. These ignimbrites shown new features which require that a reappraisal be made of emplacement mechanisms, and new diagnostic criteria be made for recognising pyroclastic types.

'Ignimbrite' is a rock body made predominantly from pumiceous material (pumice, glass shards), which may or may not be welded, and has characteristics indicating that it was emplaced as a hot and concentrated particulate flow. By concentrated we mean that the volumetric particle:gas ratio in the moving flow was relatively high; pumice density[10] and fluidisation[11] studies on non-welded flows indicate that they were little expanded beyond their observed density. We thus use the word ignimbrite in a genetic sense, more or less synonymously with ash flow.

The outstanding feature of the Taupo and Rabaul ignimbrites is that both occur in two contrasted forms (Fig. 1) having quite different relationships to the pre-existing land surface: one form occurs as a mantling layer generally less than 2 m thick which more or less completely drapes the landscape; the other form occurs in the 'conventional' way as valley-ponds having a nearly horizontal upper surface and locally attaining a depth exceeding 10 m. The name 'ignimbrite veneer deposit' is proposed for the landscape-blanketing form which connects the valley-ponds in a continuous sheet.

At the edge of the valleys the ignimbrite veneer deposit is visibly continuous with the ponded ignimbrite, and this continuity is observed at so many points that there is no doubt that both forms are part of a continuous rock body. The ignimbrite veneer deposit has practically identical grain size and constitution to the ponded ignimbrite, except that it lacks the coarsest pumice clasts, and it shows a crude layering in which the layers are 1–20 cm thick and differ from one another mainly in maximum pumice size. Locally it also contains discontinuous lenses of well rounded and well sorted pumice.

Our interpretation of the veneer deposit is that the pyroclastic flow surmounted more or less the entire landscape when spreading outwards from source, and after the passage of the main body of the flow the material drained only very incompletely into the valleys, leaving behind a mantling layer on the interfluves. This layer lacks the coarser pumice which, being concentrated in the upper or middle parts, moved on with the body of the flow. The veneer is a 'tail' deposit left in the wake of the pyroclastic flow.

The significance of an ignimbrite veneer deposit is that it is deposited from, and records the passage of, a pyroclastic flow. In the Taupo example, its presence shows that the flow surmounted hills rising more than 1,000 m above the present level of the vent.

The closest analogue to the Taupo and Rabaul ignimbrites in the literature is the Koya flow (Japan)[12] which extends to 60 km from the vent with a thickness of generally less than 1 m. The Lajes ignimbrite in Terceira (Azores)[13] reaches a thickness of 10 m at several places around the coast of the island which are interconnected by a thin layer, now interpreted as a veneer deposit, draped over the intervening country. An example of a densely welded ignimbrite is known on Fantale (Ethiopia)[14] where it clearly occurs as two forms, one as a layer 1–2 m thick spread over much of the surface of the volcano, including some upstanding lava domes, and the other ponded at the foot of the volcano. The continuity of the densely welded Skessa tuff of Tertiary age in Iceland[15], which extends over 430 km^2 with an average thickness of 6 m, appears to have more the characteristics of a veneer deposit than those of a 'conventional' ignimbrite.

Recognition of the ignimbrite veneer deposit as a distinct type requires that the diagnostic criteria for the various pyroclastic types be updated. Similarly to a pyroclastic fall deposit, it mantles the topography and has internal layering. However, it is distinguished by the rapid lateral variations in thickness and occasional cross-bedding shown by the layers, and by the local presence of laterally discontinuous pumice lenses. Like a base surge deposit[16] it shows a distinct cross-bedding, but the angle between layers is commonly less than 10°, much less than in surge deposits. Moreover, the grain-size contrast between layers is negligible compared with that in surge deposits and it also contains carbonised vegetation.

The extensive ignimbrite literature concentrates on the larger-volume and more impressive examples, those which are generally in part at least welded and have smothered the existing landscape to create their own new aggradational surface.

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* Present address: Geology Department, Imperial College, London SW7, UK.

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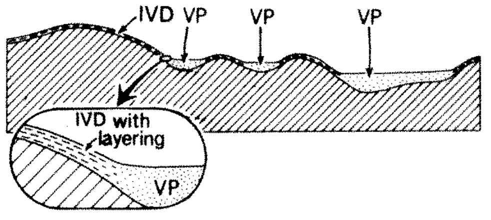

Fig. 1 Sketch showing the relationship between the landscape-mantling ignimbrite veneer deposit (IVD) and the associated valley-ponded ignimbrite (VP) as exhibited by the Taupo and Rabaul ignimbrites.

Workers in volcanic areas have therefore tended to regard the features of these big ignimbrites as characteristic of all ignimbrites; there is a reluctance to apply 'ignimbrite' or its synonym 'ash flow' to thin non-welded sheets, particularly when they mantle the topography and display a crude layering.

The word ignimbrite was originally applied to welded ash flows[17], but it has since become clear that welding of an ignimbrite is largely an accident of circumstance; it depends for example on the eruptive column height (as this determines the magnitude of the heat loss at the vent[1,18]) and on the thickness of the sheet. If 'ignimbrite' is applied in a genetic sense to rock bodies which have been emplaced by a common pyroclastic flow mechanism, then its definition should include non-welded as well as welded examples. Similarly, thin and thick, landscape-mantling and valley-ponded, variants should also be included.

The veneer deposit of the Taupo ignimbrite covers about 15,000 km^2 with an average thickness of ~1 m, and it contains roughly half of the total volume of the ignimbrite. The veneer deposit of the Rabaul ignimbrite covers well over 1,200 km^2—we did not attempt to trace it to its extremities—with an average thickness of ~2 m, and constitutes probably more than half of the total volume. The description of the Koya flow suggests that it probably consists entirely of a veneer deposit. Even the Valley of Ten Thousand Smokes ignimbrite shows a 'high sand mark'[19] which is especially well developed in the near-vent area and described as reaching 60 m above the adjacent valley-pond level towards which it slopes at 10–15°, and may be interpreted as a veneer deposit. We propose that ignimbrites form a spectrum of types, ranging from those in which the veneer deposit constitutes the entire volume, to those in which it constitutes none.

Ignimbrites such as Taupo, Rabaul (and Koya?), in which the veneer deposit constitutes a large proportion of the total volume, may be characterised as ignimbrites of low aspect ratio type. These three examples have an aspect ratio of 1:70,000, 1:7000 and ~1:200,000 respectively, and the Skessa Tuff 1:4,000. In contrast, the VTTS ignimbrite has an aspect ratio of about 1:400 and the Bishop Tuff[20] about 1:250.

It remains to consider why these ignimbrites have a low aspect ratio. It is known that the Taupo ignimbrite eruption was immediately preceded by a plinian phase in which the eruptive column, judged from the wide dispersal of the plinian pumice, was quite exceptionally high[21], and the low aspect ratio is attributed to the high momentum imparted to the pyroclastic flow when catastrophic collapse from this high column occurred and also to the high magma discharge rate. There is good evidence that the Rabaul column was similarly very high. We lack detailed information on the Koya event. In all three ignimbrites the density of the pumice is unusually low, but we think that this is more significant to the dynamics of the explosive eruption than to the mobility of the pyroclastic flows.

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Exploring pictures tactually

Lochlan E. Magee & John M. Kennedy

Division of Life Sciences, Scarborough College, University of Toronto, West Hill, Ontario, Canada M1C 1A4

Raised-line drawings of common objects can be identified by congenitally and early blind subjects[1,2] and displays of this type are being devised for use in education (for example, ref. 3). Although a teacher may think it helpful to guide a blind student's hand around one of these drawings, several theories of perception predict that the guidance would be detrimental as passive perception is generally considered to be inferior to active perception (for review see ref. 4). We show here, however, that guidance is helpful and leads to better identification than unguided exploration. We also report that the information used to identify raised-line drawings is predominantly kinaesthetic.

Our first two studies compared active and passive conditions for recognition of raised-line drawings. Although the practical application of this comparison concerns the blind, the issue can be tested using blindfolded subjects. In the first study, eight raised-line drawings of familiar objects were presented individually to 40 blindfolded students. Half of the subjects were passive, that is, the experimenter traced the index finger of the subject's preferred hand around a figure, controlling the direction, extent and speed of movement. The performance of these passive subjects excelled that of the active subjects, who determined their own exploration. The passive group successfully identified 42 drawings compared with 20 identified by the active group, a highly significant difference according to a ranked sums test ($P < 0.005$). This study has the practical implication that a blind student could profit from the guidance of a sighted instructor when first trying to identify raised-line drawings.

Our unexpected result prompted us to carry out a yoked comparison of the two types of exploration in which the movements of a passive subject's finger were matched moment by moment to those of an active subject. Yoking was accomplished with the use of a computer system and a digitised tablet on which the drawing rested. The position of a small coil surrounding the index finger of a subject's preferred hand was monitored within the area of the tablet to the nearest 0.25 mm and recorded every 200 ms to retain a detailed record of exploratory behaviour. The record of the path traced by an active subject could be 'played-back' on a television monitor situated next to the digitised tablet. The entire path was outlined by dots, each dot specifying the location of the centre of the coil at a particular time. A small cursor moved to each dot in turn, thus retracing the subject's exploratory movements.

Eight naive subjects participated as active explorers. One of 12 raised-line drawings was taped to the tablet surface as each blindfolded subject individually explored the complete set of drawings. Each subject was instructed to trace the figure in a clockwise direction, without repetition (retracing). A complete set of tracings was recorded for each of the active subjects exploring each of the 12 drawings presented in different random orders. As an example, Fig. 1 shows a copy of a tracing by an active subject.

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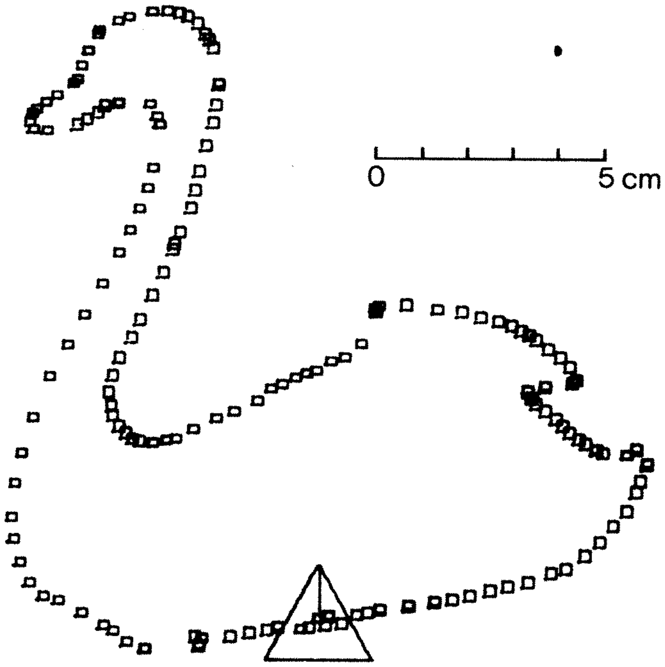

Fig. 1 A tracing of a raised-line drawing of a swan by an active subject. Each small square represents the position of the subject's finger at 200-ms intervals. Exploration began at the centre of the large triangle (not actually present as a raised-line) and proceeded in a clockwise direction. The density of squares therefore indicates the speed at which the finger was moved over each part of the display. An analogue of this figure was available on a monitor to guide the experimenter when passive subjects participated in exploring the form. All raised-line drawings for this experiment were formed by a single continuous line defining the outline of an object easily identifiable by sight. These included a drawing of a butterfly, a boat, a crown, a fish, a goblet, a claw hammer, a hand, a body, a rabbit, an umbrella, a swan and a flower.

Eight passive subjects encountered each of the drawings as presented to their active counterparts. At the same time as the recording of an active subject's performance was replayed by the monitor, the experimenter traced the passive subject's finger along the drawing. A second cursor on the monitor showed the current location of the passive subject's finger. The experimenter, watching the monitor, kept the passive subject's cursor as close as possible to the replay.

A record of the passive subject's finger locations was kept so that the degree to which the active and passive tracings departed could be determined. This involved a calculation of the distance between the active and passive subjects' positions for each interval averaged over the number of intervals needed to complete the trace. As the cursor for the passive subject normally followed the cursor for the active subject, mean deviation was computed for various time lags and a minimum value for mean absolute deviation was specified for each drawing. Averaged over the set of drawings and across pairs of subjects, the overall mean deviation was found to be 2.28 mm, at an average lag of approximately 161 ms. This value represents an average of only 0.67% difference in the areas of the two patterns. Therefore, a difference in identification rates between the two groups is unlikely to be accounted for by a difference in exposure. In agreement with the previous study, passive subjects performed better than active ones. The passive group identified 25 drawings, whereas 16 were identified by the active group, a difference significant under a Wilcoxon matched-pair signed-rank test ($P < 0.05$).

The success of passive participation readily allowed determination of the source of information subserving identification. This involved a comparison of two more groups. For one of these groups ($n = 10$), the subject's index finger was held stationary while the raised-line drawing was moved underneath the fingertip so that the twists and turns of the raised-line could be detected cutaneously unaccompanied by any movement on the subject's part. For a second group ($n = 12$), exposure to the raised-line drawings allowed for kinaesthesis but not for cutaneous input. Here, the subject's finger was moved passively around the outline of a figure where the raised line was not present. The experimenter simply used a clear plastic sheet superimposed on a pencil drawing for guidance. Only five figures were successfully identified by the 'cutaneous' group, whereas 25 figures were identified by the 'kinaesthetic' group. This difference is highly reliable according to a Mann-Whitney *U*-test ($P < 0.001$). Clearly, the ability to identify raised-line drawings does not depend on cutaneous information as it would in braille reading. It seems that information arising from limb position and limb movement is predominant. This does not mean that the raised line fails to serve a useful purpose. Obviously, the raised line is needed to guide movement during active exploration but it is on the resultant kinaesthetic information that identification of the pattern rests.

The importance of kinaesthesis suggests that the difference between active and passive performance may be accounted for by considering how initiation, execution or monitoring of movements differs in these conditions. Active subjects have the additional requirement that they must plan exploratory movements. Some of these intended movements will be translated into activity whereas others may fail to be initiated or be only partially executed. The active group may find it difficult, in memory, to separate intended movements which are acted on from those which are not. Further, the act of planning movements may draw on the limited resources of the haptic system so that less processing capacity is available to monitor and distinguish relevant from irrelevant kinaesthetic input.

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Influence of brood hosts on host preferences of bark beetle parasites

Louis H. Kudon & C. W. Berisford*

Department of Entomology, University of Georgia, Athens, Georgia 30602

The southern pine beetle, *Dendroctonus frontalis* Zimmerman (SPB) is attacked by several species of hymenopterous parasites[1,2] the most common of which also parasitise other bark beetles[3–5] including the engraver beetles *Ips grandicollis* (Eichhoff), *I. avulsus* (Eichhoff), *I. calligraphus* (Germar), *I. pini* (Say); and the eastern juniper bark beetle, *Phloeosinus dentatus* (Say). The increasing importance of pest management has focused attention on the role of their natural enemies. However, the dynamics of bark beetle parasite populations is not generally known beyond estimates of mortality caused by parasites. The hymenopteran parasite guild of *D. frontalis* may kill up to 30% of a given brood[2]. Since *D. frontalis* is cyclic in most areas with epidemics followed by very low population

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* To whom correspondence should be addressed.

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densities[1], other beetle hosts probably act as reservoirs for certain species of these parasites[3]. Conversely, during *D. frontalis* epidemics, other beetles, such as *I. grandicollis* and *P. dentatus* may compete as alternate hosts. Information on preferences for *D. frontalis* and other associated bark beetles is necessary to understand the interactions of this host–parasite complex. We report here the results of a study designed to determine if several known *D. frontalis* parasites display any host preferences among *D. frontalis, I. grandicollis* or *P. dentatus.*

Infestations of *D. frontalis, I. grandicollis* and *P. dentatus* were located in Camden, Clarke and Oglethorpe Counties, Georgia. Bolts, ~0.5 m long and 20 cm in diameter, that were uniformly infested predominantly with late instar larvae, were cut as trap bolts. Camors and Payne[6] showed that *Heydenia unica* Cook and Davis prefers third instar larvae of *D. frontalis*, and many other parasites have similar preferences (Berisford *et al.* unpublished data). Lengths of the trap bolts were adjusted according to diameter, to maintain approximately 3,000 cm^2 of bark surface. Each trap bolt was infested with only one species of bark beetle. *D. frontalis* and *I. grandicollis* trap bolts were cut from loblolly pine (*Pinus taeda* L.) and *P. dentatus* trap bolts were from eastern red cedar (*Juniperus virginiana* L.). Concurrent with the above collections, bolts of similar size which had beetle pupae or callow adults with parasites that were about to emerge were cut as a source of the parasites.

All the bolts were removed to University of Georgia School of Forest Resources Whitehall experimental forest near Athens, Georgia. Traps constructed from 30 × 20 cm aluminium metal screening (16 × 18 mesh) were affixed to the trap bolts which contained third instar larvae and the screens were coated with a thin layer of 'Stikem Special'. The trap bolts were then suspended by hooks from wooden frames inside 6 × 6 × 6 ft (1.83 m) saran screen cages (32 × 32 mesh). The cages were erected under a light pine overstory. Source bolts were placed at the bottom of the cages. Sunlight was blocked from the cages by a sheet of 100 μm thick black plastic draped over the top. This prevented the parasites from orientating to the light at the top of the cages. Four trap bolts of each beetle species involved in the experiment, and four source bolts, all infested with the same beetle species were used in each cage. Uninfested control bolts were used in preliminary experiments but discontinued when they were found to be unattractive to parasites. All possible two- and three-host combinations were tested except *P. dentatus* and *I. grandicollis* alone.

The sticky traps on the infested bolts were inspected daily for up to 2 weeks depending on parasite emergence from the source bolts. Parasites were removed from traps, cleaned in petrol and saved for identification. Trap bolts were checked as long as parasites emerged from source bolts or until larvae in trap bolts began to pupate. Responses of parasites to beetle hosts from which they were reared was compared using the normal approximation to the binomial in a one-tailed test.

During 25 field cage experiments, 681 parasites comprising six species were trapped. Parasites trapped included *Heydenia unica* Cook and Davis, *Roptrocerus xylophagorum* (Ratzeburg), *Coeloides pissodis* (Ashmead), *Dendrosoter sulcatus* Muesebeck, *Cecidostiba dendroctoni* Ashmead, *Spathius pallidus* Ashmead and *Spathius impus* Matthews.

Some of the parasites are somewhat host-specific but were included for comparison. They were not considered in the overall analysis. *S. pallidus* parasitises *I. grandicollis*[5] and *D. frontalis*[2] but not *P. dentatus*. However, *P. dentatus* is parasitised by *S. impus*[7] which is apparently host specific to *Phloeosinus* spp., *D. sulcatus*, although a frequent parasite of *Ips grandicollis* and *D. frontalis*[5] probably does not parasitise *P. dentatus*. The orientation of these host-specific parasites to their proper host is indicative of the efficacy of the experimental technique. *Cecidostiba dendroctoni* has been suspected of parasitising *I. grandicollis*[8]. All *Cecidostiba* reared from *I. grandicollis* came from trees removed from the same site in Camden County, Georgia. Apparently, in that area, *C. dendroctoni* is a relatively common parasite of *I. grandicollis. H. unica, R. xylophagorum* and *C. pissodis* attack all three host beetles[2–5,9,10].

Parasites which emerged from a given host returned to that host in significantly greater numbers (Fig. 1). Parasites reared from *D. frontalis* and exposed to *P. dentatus* and *D. frontalis* as

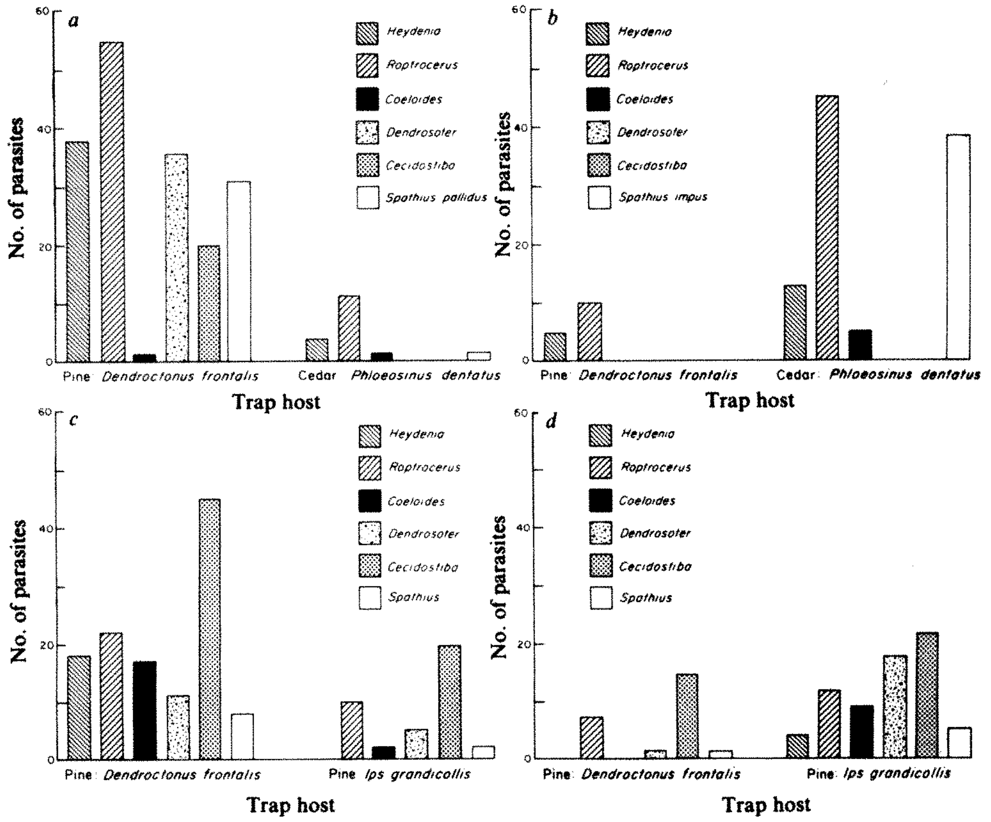

Fig. 1 Numbers of hymenopterous parasites collected from trap bolts in field-cage experiments. *a*, Parasites reared from *Dendroctonus frontalis* and trapped on logs containing late instar larvae of *D. frontalis* and *Phloeosinus dentatus* during simultaneous exposure to the parasites. *b*, Parasites reared from *Phloeosinus dentatus* and trapped on logs containing late instar larvae of *P. dentatus* and *D. frontalis* during simultaneous exposure to the parasites. *c*, Parasites reared from *Dendroctonus frontalis* and trapped on logs containing late instar larvae of *D. frontalis* and *I. grandicollis* during simultaneous exposure to the parasites. *d*, Parasites reared from *Ips grandicollis* and trapped on logs containing late instar larvae of *I. grandicollis* and *D. frontalis* during simultaneous exposure to the parasites.

hosts showed an almost tenfold preference for *D. frontalis* (Fig. 1*a*). When parasites reared from *I. grandicollis* or *D. frontalis* were given a choice of these two pine beetles, the split was not so pronounced, with about three times as many parasites returning to the 'parent' host (Fig. 1*c* and *d*). This difference is probably due, in part, to parasites orienting to the tree host (pine vs cedar). *Ips* spp. parasites may manifest differential responses to different tree hosts[11], but both *I. grandicollis* and *D. frontalis* were in loblolly pine in our study. Differences in response in this case probably could not be attributed to the host tree.

Parasite totals for each combination of hosts were shown to be significant at the 5% level except the three host combination with parasites reared from *Ips*. Although statistically non-significant, probably due to the relatively small sample, more parasites selected bolts infested with *I. grandicollis*. These data were significant (5% level), however, when combined with the two host combinations (*Ips* and *Dendroctonus*, with parasites reared from *Ips*). Most counts for individual parasite species from each combination also showed significantly stronger attraction to the species of host from which they were reared.

This host preference phenomenon is an apparent example of Hopkins' host selection principle[12]. Although the principle was proposed for phytophagous insects and has been demonstrated[13,14], exceptions have been noted[15,16]. Our study is one of few apparent demonstrations of Hopkins' host selection principle involving parasites of animals[14,17] and the first for parasites of bark beetles.

Parasites which are not host specific tend to attack the host on which they were reared but they retain the ability to use other hosts if the preferred host is unavailable. This behaviour is advantageous to the parasites in that they may use all potential hosts more efficiently by switching to relatively more abundant alternate hosts when populations of preferred hosts decrease. This phenomenon may also increase prey diversity[18]. The switching mechanism should be particularly helpful to species with relatively low host-finding efficiencies. The most common parasite in our study, *R. xylophagorum*, is unable to locate potential hosts in low numbers proportional to the number located when hosts are abundant[3] but its ability to attack a variety of hosts apparently allows it to maintain substantial populations.

The impact of the parasite guild of the southern pine beetle may be affected by numbers of alternate hosts. Parasites may switch from other hosts during expansions of *D. frontalis* populations if *D. frontalis* becomes much more abundant than the 'preferred' host. Therefore, the host selection mechanism serves to concentrate the parasites on the epidemic host after a period of time. As the epidemic populations decline, the parasites can accept other hosts as they become relatively abundant, thereby reducing the impact of parasites on declining host populations.

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Mate choice increases a component of offspring fitness in fruit flies

Linda Partridge

Department of Zoology, University of Edinburgh, West Mains Road, Edinburgh, UK

The authors of two influential accounts of the genetic consequences of mate choice consider that animals cannot produce fitter offspring by mating with a fitter than average individual[1,2]. Their main reason for this is that in a population at genetic equilibrium any genetic variation which affects fitness should not be heritable[3]. Other accounts have suggested ways in which mate choice might improve offspring fitness[4]. Data are now presented which show that one component of offspring fitness can be increased by mate choice in *Drosophila melanogaster.* The component of fitness measured was intraspecific competitive success during the part of the life history from first instar larva to adult fly.

The flies used were an outbred stock collected in Dahomey in 1970 and maintained in approximately constant conditions in a population cage at the Department of Genetics, Edinburgh University. Two different experimental comparisons were made. In the first (experiment 1), the offspring of inseminated adult females which had mated in the population cage where many males were present were compared with offspring of females collected from the cage as virgins and mated with a randomly chosen male from the cage. The comparison was made by taking first instar larvae from eggs laid by the 'choice' and the 'no choice' females and competing each set of larvae against an equal number of larvae of Dahomey stock made homozygous for a fourth chromosome bearing the recessive mutant *sparkling.*

Table 1 The competitive success of offspring of flies which had chosen (choice) or not chosen (no choice) their mates

		% Wild type		No. of
		Mean	Variance	vials
First replicate	Choice	51.1	7.1	23
	No choice	48.9	8.7	18
	$t = 2.45$	$P < 0.02$		
Second replicate	Choice	49.8	2.2	21
	No choice	48.1	5.8	14
	$t = 2.45$	$P < 0.02$		

Experiment 1: The experimental details were as follows. Adult male and inseminated female flies were collected from the population cage using a pooter. After anaesthetisation with carbon dioxide, 100 males and 100 females were taken and placed individually in shell vials with food. Eclosing virgin females (100) were then collected from bottles in the population cage and placed individually in the 100 vials containing single males. After 2 days the 'no choice' females were separated from the males, and females of both groups were then set up in batches of 10 and allowed to lay eggs on 10% agar medium with a small amount of yeast present. At the same time, other batches of 10 females were set up using the Dahomey *sparkling* stock. All females were allowed to lay for 24 h before being moved on to fresh agar, and after a further 16 h first instar larvae were collected from the surface of the original agar using a paintbrush. The larvae were then placed in two groups of vials. Vials in the first group each contained 200 larvae from the 'choice' females and 200 *sparkling* larvae; vials in the second group each contained 200 larvae from the 'no choice' females together with 200 *sparkling* larvae. The vials were set up in this way over a period of 10 days, repeatedly taking offspring from the same females which used stored sperm during this period. It was therefore not known how many larvae each female contributed to each vial. The 400 larvae in each vial were allowed to compete and pupate, and the number of wild-type and *sparkling* flies emerging from each vial was recorded. Competitive success was measured as the percentage of wild-type (as opposed to *sparkling*) flies emerging from each vial. Significance levels are on a two-tailed *t*-test.

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Table 2 The competitive success of offspring of females of the same age which had chosen (choice) or not chosen (no choice) their mates

	% Wild type		No. of vials
	Mean	Variance	
Choice	50.45	2.0	28
No choice	49.44	2.0	32
$t = 2.72$	$P < 0.01$		

Experiment 2: Adult flies were collected from the population cage using a pooter; after anaesthetisation with carbon dioxide the females were discarded, 100 males were placed individually in shell vials with food and 300 were placed in a single bottle with food. Eclosing females were then collected from bottles in the cage, and 100 were placed in the bottle containing 300 males and 100 individually in the 100 vials containing single males. The flies were left for 2 days, after which laying pots and competition vials were set up as in experiment 1. Competitive success was measured as the percentage of wild-type flies emerging in each vial. Probability level is on a two-tailed *t*-test.

Larvae were allowed to compete in vials which contained the same food medium as that used in the population cage, and the number of wild-type and *sparkling* flies emerging from each vial was recorded. The point of this experimental procedure was to ensure that the wild-type parent flies were exactly matched for nutrition and numbers of anaesthetisations, and that the larvae were competing under circumstances similar to those encountered by larvae in the population cage, both with respect to the nature of the food and the genotype of the competitors. The experiment was done twice, and the results, shown in Table 1, indicate that in both replicates the percentage of wild-type flies emerging was slightly, but significantly, higher when the parents of those flies had chosen their mates.

Because the 'choice' flies in experiment 1 were collected at an older age than the 'no choice' females, experiment 2 was carried out to control for any effect of maternal age. First instar larvae were collected from parents of the same age who had either chosen or not chosen their mates, and the larvae were again competed against *sparkling* larvae. The results are shown in Table 2. Again, the percentage of wild-type flies emerging was larger if the parents of those flies had chosen their mates.

The results show that matings between randomly chosen pairs of flies produce offspring with lower survival between larvae and adult than matings where mate choice can occur. The results do not show whether the overall fitness of the offspring is affected, because some other component of fitness may be negatively correlated with the one measured.

The result could be produced by various genetic mechanisms. First, fitness may indeed be heritable, possibly as a consequence of mutational load[5]. If this were the case, the results could be produced in two ways. (1) Fitter flies may be better at detecting or obtaining access to mates. Members of the other sex could then mate with a fly that was successful in some sort of competition with members of its own sex. (2) Flies may be able to detect heritable fitness in members of the other sex. Fitter flies could then be actively chosen as mates. Both possibilities would suggest that all flies should prefer the same sorts of mates. Alternatively, flies with high levels of heterozygosity may have high fitness. In this case, flies might produce fitter offspring by mating with individuals genetically unlike themselves, so that their offspring will have higher levels of heterozygosity. This last possibility would imply that genetically dissimilar flies would show different patterns of mate choice.

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Larval competition and brood sex ratios in the gregarious parasitoid *Pachysomoides stupidus*

John Pickering

Department of Entomological Sciences, University of California,
Berkeley, California 94720

The haplodiploid genetics of the Hymenoptera, by which males and females develop from unfertilised and fertilised eggs, respectively, give rise to certain asymmetrical coefficients of relatedness among family members[1-3]. These asymmetries, and predictions concerning the sex ratio that are based on them[2,4,5], make the Hymenoptera ideal for the study of social behaviour. Work has focused on the more highly social groups of ants, bees and wasps[6-10]. Although there has been considerable work on the parasitoid Hymenoptera with respect to sex ratio theory[11-15], no study has examined the social interactions of gregarious parasitoids in the light of inclusive fitness theory[1]. I discuss here conflict among family members of a parasitoid wasp over the allocation of host resources, and argue that males are the more altruistic/less selfish sex. This provides a further explanation for hymenopteran males being generally smaller than females and for investment ratios being biased towards females[4,16]. Conflict is predicted between mothers and their daughters over the allocation of resources to the sexes. The observed brood sex ratios are interpreted as a means by which egg-layers equalise investment in the sexes[17].

Pachysomoides stupidus (Hymenoptera, Ichneumonidae) is a parasitoid of the social wasp *Polistes canadensis* (Hymenoptera, Vespidae). A female alights on a host nest and lays her eggs within a cell containing an early-stage pupa. Up to 31 parasitoids develop together on a host pupa. The larvae feed externally, starting with the pupa's abdomen and finishing with its head. Before a host is consumed, some larvae may be excluded by their siblings from feeding. The wall of the cell surrounding the host constrains the larvae. When the larvae are small there is room for all of them to feed. As they move forward, when their girths fill the cross-sectional area of the cell, one larva loses access to the host while the others continue to feed. In larger groups this process is repeated. For example, at pupation in broods of 13, only six or seven are near the host's remains. The individuals farther from the remains are generally smaller.

Between August and October 1977, 56 *P. canadensis* nests with pupae were collected around Barro Colorado Island in Panama. The 70 broods of *P. stupidus* were allowed to develop in the laboratory. They came from 26 nests. Whenever possible the sex and position of brood members were recorded. Complete sex ratios were obtained for 50 broods. In the other broods 46 individuals were not sexed because of larval death, eclosion before nest capture, escape or hyperparasitism. The aggregate sex ratio for all 70 broods was close to 1:1 (292 males:308 females).

Dry weight was used as a measure of size. Males, on average, were smaller than females (means of 2.16 and 2.76 mg, respectively); however, the modal size of both sexes was equal (2.2 mg). Ranges were 0.4–5.0 mg for males and 0.5–5.7 mg for females. The large, 14-fold range in size reflects among and within brood differences. Smaller individuals came from larger broods with, on average, less food per individual. In any given brood the males were generally smaller than the females and usually pupated at the end farthest from the host's remains. Of

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42 non-unisexual broods that were weighed, the heaviest individual was female in 37 and male in three; two contained a male and female of equal weight. The smallest individual was male in 33 and female in nine. Of the viable individuals for which position was known, 41 males and 155 females pupated at the host's head-end of the cell, and 95 males and 28 females at the abdomen-end.

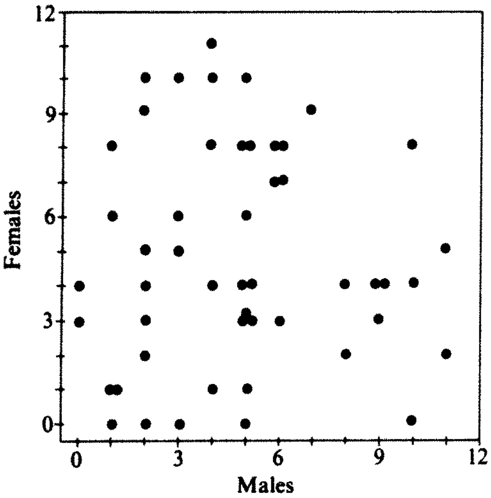

Fig. 1 Number of males and females in broods of *Pachysomoides stupidus*. The individuals from 50 complete broods reared in 1977 were sexed. Brood size ranges between 1 (a single male) and 18 (10 males and eight females), with a mean of 9.5 and a mode of 13. There are 238 males and 239 females in this sample. The distribution of sex ratios within broods was tested for overdispersion by using the statistic $D = \Sigma|M - F|$, where M is the number of males and F is the number of females within a brood, and the summation is over all broods. The sampling distribution of D was obtained by simulation. Males and females were assigned with equal and independent probabilities to 50 artificial broods of the sizes observed in the real sample, and the corresponding value of D was computed. This procedure was repeated 10,000 times. The resulting distribution of D values ranged between 75 and 171, with a mean of 119 and a standard deviation of 13.2, and was slightly skewed right. The value of D corresponding to the 50 real broods represented is 177. Thus the observed brood sex ratios are significantly overdispersed ($P < 0.0001$).

This competition is interesting in the light of inclusive fitness theory[1] because asymmetrically related brothers and sisters were developing where access to food by some siblings could be blocked by others. With haplodiploidy, where females develop from fertilised eggs and males from unfertilised eggs, asymmetries in the coefficients of relatedness (b) arise[2,3]. Assuming no inbreeding, a male is more related to his sister ($b = 1/2$) than she is to him ($b = 1/4$). Sisters are related to each other by more ($b = 3/4$) than are brothers ($b = 1/2$). A mother is equally related to sons and daughters by $b = 1/2$. That males are usually smaller than females when food is limited, and yet that males are nearly as large as females if given adequate food, is explained in part by the asymmetry in relatedness between brother and sister. If the cost (C) to an altruist and benefit (B) to the recipient of an act are ranged as the change in the proportion of progeny produced, then the threshold for selection to favour a brother helping a sister ($C < B/2$) is lower than for a sister helping a brother ($C < B/4$). By similar reasoning, a female will be selfish to a brother at a higher cost (to recipient) to benefit (to actor) ratio than he will be to her; thus she is more likely to push him away from the food than vice versa. It is not known whether males feed less because they are altruistic or because females are selfish. However, female selfishness is suggested in that male mortality is greater and in that males are more likely to have deformed antennae, legs or wings. Eleven of 308 females were dead compared with 25 of 292 males.

Conflicts are expected between a mother and her offspring, particularly her daughters, over the allocation of host resources. The distribution of adult sizes produced from a host that would give a mother her highest inclusive fitness is not necessarily the distribution that would give an individual brood member its highest inclusive fitness. Let a certain amount of food, if eaten by a male, increase his expected reproductive success by B_m and, if eaten by a female, increase her expected reproductive success by B_f. Sisters are selected to allow each other to feed in preference to brothers more often than their mother would be selented to allow them to do so. Applying the appropriate coefficients of relatedness, sisters favour a female feeding whenever $3/4B_f > 1/4B_m$, while a mother favours a daughter feeding only when $1/2B_f > 1/2B_m$. Thus a conflict between a mother and her daughters occurs when $3B_f > B_m > B_f$. There is no similar conflict between a mother and her sons: all are favoured if a female feeds when $B_f > B_m$ and a male feeds when $B_m > B_f$. Individuals of both sexes are selected to be more selfish than selection on the mother favours[18,19]. When individuals benefit from eating the food themselves by B_s, a male is selected to eat in preference to his brother when $B_s > B_m/2$ and to his sister when $B_s > B_f/2$; a female is selected to eat in preference to her brother when $B_s > B_m/4$ and to her sister when $B_s > 3/4B_f$. A mother is selected to allow an individual to feed when $B_s > B_m$ and $B_s > B_f$. Because the mother is absent, it appears that the parasitoid hosts are divided up by the offspring.

The sexes were not distributed among broods at random but were clumped within broods. In general some broods contained more females than would be expected and others more males (Fig. 1). This skewing of brood sex ratios increases the average coefficient of relatedness within sibling groups and reduces contact between asymmetrically related brothers and sisters. As females of many Hymenoptera species accurately control the sex of their offspring by controlling fertilisation[13,14,20], female *P. stupidus* may have been selected to adjust brood sex ratios to reduce the number of situations in which there is a conflict with offspring over the distribution of resources. Daughters are selected to take a greater proportion of the resources than their mother is selected to give them[4,17,21-23]. The observed distribution of brood sex ratios suggest that more resources went to males than would have, had broods been comprised of equal numbers of each sex.

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Chemical modification reduces the conductance of sodium channels in nerve

Frederick J. Sigworth*

Department of Physiology, Yale University School of Medicine,
New Haven, Connecticut 06510

Bruce C. Spalding†

Department of Physiology and Biophysics, University of Washington
School of Medicine, Seattle, Washington 98195

Tetrodotoxin (TTX) and saxitoxin (STX) are extremely potent poisons that prevent nerve and muscle cells from producing action potentials by blocking sodium channels[1,2]. If the channels are modified by reagents that act on carboxyl groups, however, both the binding of these toxins[3,4] and their effect on the action potential[5] are reduced. One such reagent, trimethyloxonium ion (TMO) converts channels into a form that is not blocked by TTX concentrations 10^5 times greater than its normal K_d (ref. 6). Most such chemical modifications of sodium channels also reduce the measured membrane sodium current, but it has not been known whether such reductions were due to a change in the number of channels, in permeability properties, or in gating properties. We now report that TMO-modified, TTX-resistant sodium channels have a smaller single-channel conductance (γ) with a more linear instantaneous current–voltage relationship than that of normal channels, and that the measured reduction in γ accounts for all of the decrease in sodium current after TMO treatment. This change in sodium channel permeability properties can be explained by the removal of a fixed negative charge near the outside of the channel.

Single nodes of Ranvier from the frog *Rana pipiens* were voltage–clamped by the method of Dodge and Frankenhaeuser[7]. Single-channel current amplitudes were estimated by ensemble fluctuation analysis[8] from the mean current variance in sets of 100–500 current records from identical depolarisations. The analysis yielded estimates for the single-channel current i and the total number of channels N available for conduction. The single-channel chord conductance γ was calculated from values of i obtained at membrane potentials near 0 mV.

Brief treatment of nerve fibres with TMO ($(CH_3)_3O^+$), reduced the size of the sodium currents in Ringer solution, and the currents were reduced further when 200 nM TTX was added to block nearly all of the unmodified channels (Fig. 1). As discussed below, the reduction in current after TMO treatment was completely accounted for by the conversion of channels with a normal single-channel conductance ($\gamma = 7.1$ pS) to TTX-resistant channels having a reduced single-channel conductance ($\gamma = 2.4 \pm 0.3$ pS) (Fig. 2). Unfortunately, the fluctuation analysis technique does not allow us to distinguish between a homogeneous population of channels and a population having a spectrum of γ values, but for the sake of simplicity we consider both the normal and modified channel populations to be homogeneous.

Table 1 compares the values of γ, N and peak current I_{peak} obtained with 70 mV depolarisations from rest before and after TMO treatment. Estimates for the number of unmodified and modified channels open at the peak, N_{open}, are also given. Values of N_{open} for each component were obtained from the ratios of the peak current to the corresponding single-channel current value. The total number of channels open at the peak is seen to be the same (within 10%) before and after TMO treatment. This indicates that the only effect of the TMO treatment appears to be conversion of normal to TTX-resistant channels; the total number of available channels remains constant. These comparisons of numbers of open channels rely on the observation, described below, that channel gating is not appreciably changed by TMO treatment. Similar values for the fraction of channels modified by TMO are obtained from comparisons of the total number of unblocked channels (N) estimated from the current fluctuations. These estimates of N are not based on many assumptions about channel gating but tend to be less reliable than γ values because of their increased sensitivity to the magnitude of background noise[8].

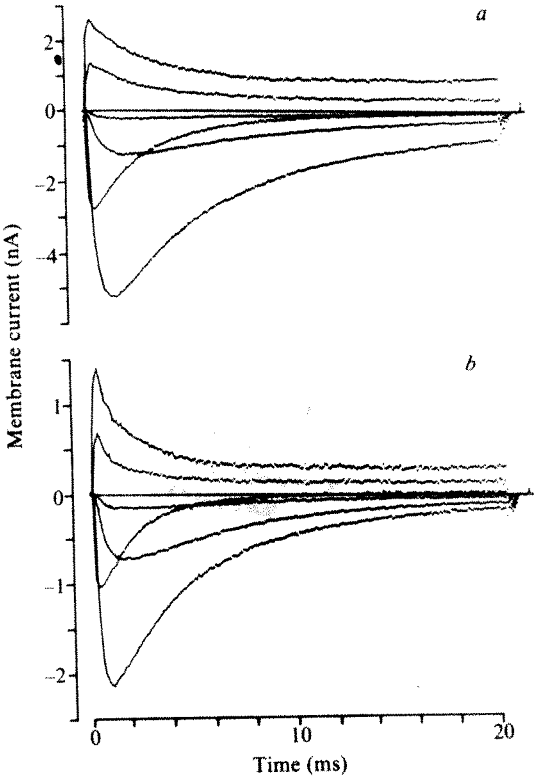

Fig. 1 Comparison of currents through *a* normal and *b* TMO-modified, TTX-resistant sodium channels in fibre 39 during 20 ms depolarisations to −45, −35, −15, 25, 85 and 125 mV from the holding potential of approximately −75 mV. Nerve fibres were treated with TMO as described in Table 1; the external solution in *b* contained 200 nM TTX. Fibres were voltage-clamped[7] at 5 °C in a Ringer solution containing 100 mM Na^+ as well as 20 mM tetraethylammonium ion (TEA) to block potassium current[9,10]. TEA was also present in the end pools. (TMO treatment reduced potassium current by less than 20%, and did not change its susceptibility to block by TEA.) A 30 ms prepulse to −105 mV preceded each depolarisation in *b*. Leakage and capacitance currents were subtracted using scaled current records obtained with 40 mV hyperpolarising pulses. The currents were signal-averaged and plotted with different vertical scales.

To determine whether TMO treatment changed the gating of sodium channels, we observed the voltage dependence of activation of the TTX-resistant currents. This voltage dependence corresponded, within 4 mV, to that for currents in the fibres before modification. The shorter activation and inactivation time constants seen in the TTX-resistant currents of Fig. 1 almost certainly arose from a small (2 °C) temperature rise during the experiment, because the change was not consistently seen, and the voltage dependence of the activation and inactivation time constants was also essentially unchanged.

Although the sodium current reversal potential was unchanged (within 4 mV) by TMO treatment, the TTX-resistant currents showed relatively larger outward currents at large depolarisations. We believe that this deviation is due to a change in the permeability properties of open channels. The instantaneous current-voltage (I–V) relationship, generally assumed

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* Present address: Max Planck Institut für Biophysikalische Chemie, D-3400 Göttingen, Postfach 968, FRG.

† To whom reprint requests should be addressed at: Dept of Physiology, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642.

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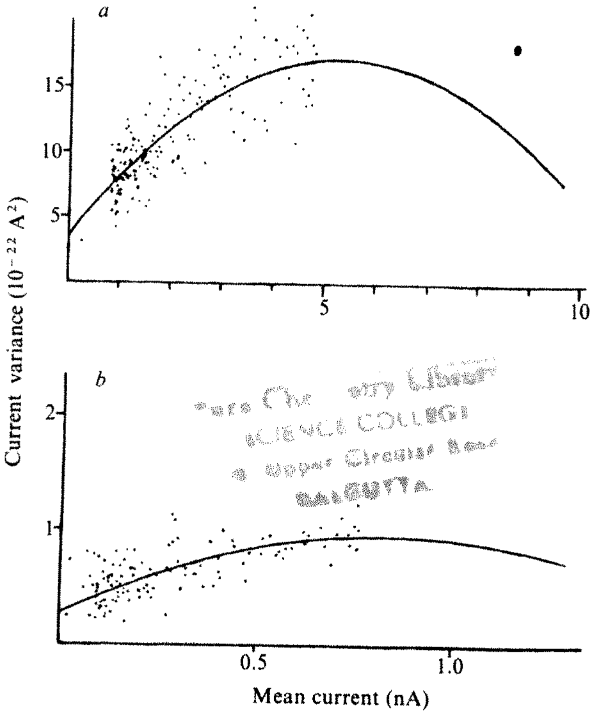

Science College Upper Circular Road Calcutta

Fig. 2 Variance–mean current plots in normal and TTX-resistant sodium channels from fibre 43. Current variance and mean current values from depolarisations to −15 mV were obtained as described in Table 1. Smooth curves were obtained by fitting the points with the relation $\sigma_I^2 = \gamma(E - E_{Na})I - I^2/N$ after correction for background noise. Scales have been chosen so that a conductance of 5 pS corresponds to a slope of one in each part of the figure. The shallower slope in *b* reflects the smaller value of γ in TMO-modified channels. *a*, Before TMO treatment; the curve was drawn taking $\gamma = 7.0$ pS and $N = 20{,}800$. *b*, After TMO treatment and in the presence of 200 nM TTX; the curve was drawn taking $\gamma = 2.6$ pS and $N = 9{,}300$.

to reflect the ion permeation properties of the channel at a constant state of the gating system[11], is plotted together with estimates of *i* at various potentials in Fig. 3. The instantaneous *I–V* relationship is less curved for the TTX-resistant currents than for the control. The close correspondence between the voltage dependence of the instantaneous current and the single-channel current estimates, like that already seen in untreated fibres[8], suggests that the modified channels have only one non-zero conductance level. Assuming this to be the case, we have scaled the instantaneous current curves in Fig. 3 using the single-channel current estimates, and identify the curves with the current-voltage relationship of single open channels.

We conclude that TMO modification of sodium channels changes the permeability properties of the channels, but leaves the gating properties essentially unchanged. The changes in single-channel conductance and rectification resemble those observed by Appel *et al.*[12] in hybrid channels formed from *O*-pyromellityl gramicidin A (OPG) and gramicidin A (G). They attributed the lower conductance and rectification of normal G–G channels, relative to hybrid OPG–G channels, to the absence of the electrostatic effects due to the negatively charged pyromellityl moiety. We expect that TMO removes a negative charge in the vicinity of the sodium channel by the esterification of a normally ionised carboxyl group. Normally, the negatively charged carboxyl group would increase the local cation concentrations near the outside of the sodium pore above that of the bulk solution. The modified channels, lacking this charge, would have a lower local concentration and would exhibit reduced conductance and rectification.

This view is supported by the observation that the effect of the hypothesised changes in the local potential and ion activities at the membrane surface may be partially simulated by suitable changes of the bulk solution potential and ionic activities. As shown in Fig. 3, the substitution of 80% of the sodium by impermeant tetramethylammonium (TMA) ions produced single-channel currents in an untreated fibre similar in size and voltage-dependence to the toxin-resistant currents in standard Ringer solution. This simulation is not exact because the concentrations of the other ions were not also suitably changed, and because TMA, although it is better than other ions in this regard, may interfere with sodium ion permeation.

In another experiment, we investigated the relationship between the site at which TMO acts to reduce γ and the toxin binding site by pretreating fibre 105 with 200 nM TTX and then treating it with TMO in the presence of TTX. After a 10 min wash in toxin-free Ringer, peak sodium current returned to 40% of its original value and fluctuation analysis revealed no decrease in γ (Table 1). Following this treatment, application of 200 nM TTX reduced sodium current to undetectable levels. Apparently, TTX prevents TMO from reaching the site at which it acts to reduce γ. This experiment also shows that the decrease in γ is not due to nonspecific action of TMO, buffer solution, or the products of TMO hydrolysis (methanol and dimethyl ether). Furthermore, other experiments[6] show that at least 80% of the sodium conductance returns on washing after treating a frog skeletal muscle fibre with TMO in the presence of STX, indicating that TMO treatment does not obstruct the movement of toxin between its receptor and the bulk solution. It seems likely that toxin resistance and reduced γ are both due to TMO action at the toxin binding site, although the possibility that TMO may act to reduce γ at a different site which is inaccessible in the presence of bound toxin cannot be ruled out.

Although in aqueous solution TMO reacts preferentially with carboxyl groups, it should be noted that the possibility that the effects we describe are due to reaction with other functional groups cannot be completely excluded. The specificity of TMO has been discussed more fully elsewhere[6].

Reversible reductions in γ have previously been observed at low *p*H (ref. 8) and in the presence of Ni^{2+} (ref. 13). Small reductions in γ have also been observed in sodium channels with modified inactivation[13]. However, this is the first report of a chemical modification of sodium channels resulting in a large change in γ with essentially no change in channel gating. It will

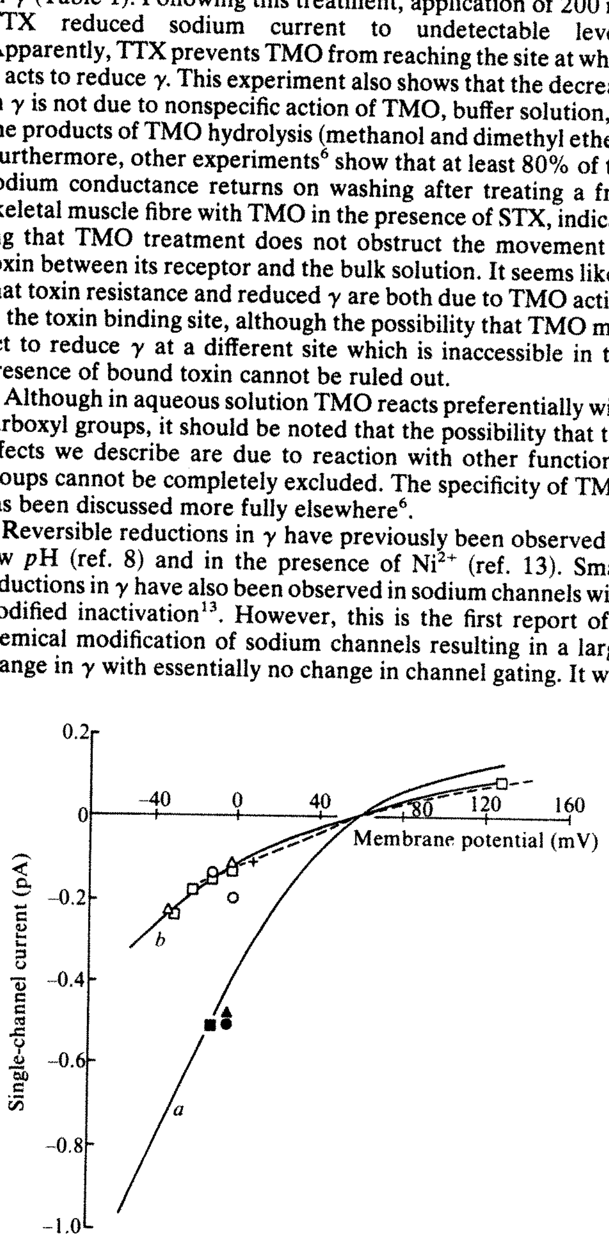

Fig. 3 Instantaneous current–voltage relations (curves) from fibre 43 and single-channel current estimates (symbols) from the same fibre (□, ■) and from fibres 38 (○, ●) and 39 (△, ▲). Filled symbols and curve *a*: normal currents, before modification with TMO; open symbols and curve *b*: TTX-resistant current; (+) and broken curve: normal currents before TMO modification from fibre 43 in one-fifth normal Na^+ (20 mM), shifted by 36 mV to superimpose the reversal potentials. The instantaneous current values, obtained at the test potential after a 1-m activating pulse to +45 mV, were scaled to fit the single-channel current values. The scaling factor is equivalent to the number of channels open at the end of the activating pulse.

Table 1 A comparison of sodium conductance before and after TMO treatment

Fibre	Treatment	N	γ (pS)	I_{peak} (nA)	Calculated N_{open} Unmodified	Calculated N_{open} Modified
38	Before TMO	22,000	7.3	7.0	13,900	—
	After TMO, no TTX	*	*	5.5	11,100	3,500
	After TMO, 100 nM TTX	7,000	3.1	0.6	—	3,500
39	Before TMO	41,000	7.2	12.0	25,600	—
	After TMO, no TTX	*	5.1*	6.5	10,300	13,100
	After TMO, 200 nM TTX	22,000	2.1	1.9	—	13,100
43	Before TMO	21,000	6.9	6.0		
	After TMO, 200 nM TTX	10,000	2.3	0.8		
105†	Before TMO + TTX	42,000	6.8	7.4		
	After TMO + TTX, no TTX	17,000	7.5	2.9		

Nerve fibres were treated with TMO by quickly dissolving the fluoborate salt (20 mM for fibre 38, 40 mM for fibres 39 and 43) in cold buffer solution (80 mM morpholinopropane sulphonic acid, MOPS) and applying this solution to the preparation. After 10 min the solution was washed out with Ringer solution. TMO reacts rapidly with water, so the concentration of reactive species that actually comes into contact with the membrane could be as little as one-half the values quoted. The tabulated values of N and γ were obtained by ensemble fluctuation analysis. A series of 100–500 sodium currents from repeated identical depolarisations was recorded, I, calculated by signal averaging, was subtracted, and the variance σ_I^2 was calculated for each point in groups of four digitised records. After a correction for background noise, the functional relationship between the variance and mean current was fitted by the quadratic function predicted by binomial statistics. For a homogeneous population of N independent channels having currents of 0 or i, the current variance is given by $\sigma_I^2 = iI - I^2/N$. The values of γ were calculated as the chord conductances, $\gamma = i/(E - E_{Na})$ where E, the membrane test potential, was chosen to be near 0 mV. E_{Na} is the reversal potential for current in sodium channels, which averaged 54 mV in these experiments. I_{peak} is the peak inward sodium current observed during a depolarisation to $E = -5$ mV, preceded by a 30 ms prepulse to −105 mV. N_{open}, the number of channels open at peak I_{Na}, was calculated for fibres 38 and 39 from measured values of I_{peak} and the corresponding γ values using the relation $N_{open} = I_{peak}/\gamma(E - E_{Na})$. The contribution of unmodified channels to the value of I_{peak} after TMO, in Ringer solution without TTX, was estimated by the difference between the I_{peak} values with and without TTX. The number of open, unmodified channels after TMO was therefore calculated as $N_{open} = (I_{peak,noTTX} - I_{peak,TTX})/\gamma(E - E_{Na})$, where the γ value from the untreated fibre was used.

* Only apparent values of N and γ can be measured after TMO treatment in the absence of TTX, since two populations of channels are present. The apparent γ value is an average of the γ values of each population, weighted by the current due to each population; the interpretation of apparent N values is less simple and these values have been omitted from the table.

† Fibre 105 was pretreated with 200 nM TTX and then treated for 10 min with 40 mM TMO dissolved in buffer solution containing 200 nM TTX. The values 'after TMO + TTX' were measured following a 10 min wash in toxin-free Ringer.

be interesting to see if naturally occurring toxin-resistant channels have properties similar to those of TMO-modified channels.

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Met-enkephalin circulates in human plasma

Vicky Clement-Jones, P. J. Lowry, Lesley H. Rees & G. M. Besser

Departments of Endocrinology, Chemical Pathology and Chemical Endocrinology, St Bartholomew's Hospital, London EC1, UK

The physiological roles of Met-enkephalin and Leu-enkephalin[1] are still unknown. They may act as neurotransmitters in the central and peripheral nervous sytems[2]. Met-enkephalin has been detected in several species in a variety of tissues including brain, spinal cord and gut using bioassays, opiate receptor assays and radioimmunoassays (RIA)[3–8]. It has also been detected in human gut immunocytochemically and in human brain and cerebrospinal fluid by opiate receptor assay and RIA[9–11]. However, all reported assays show some degree of cross-reaction with Leu-enkephalin and unequivocal differentiation between the two enkephalins and the larger endorphins has not always been possible. Thus the existence of Met-enkephalin in human tissues and fluids remains in doubt. Using a highly specific RIA, we have now obtained evidence that Met-enkephalin-like material circulates in the plasma of normal subjects and may be secreted by the adrenal gland. Chromatographically the material exists in plasma mainly as the intact pentapeptide and not as the biologically inactive degradation product Gly-Gly-Phe-Met as would be expected from metabolic studies[12,13].

Although bioassays and opiate receptor assays have the advantage of measuring biological and related activities they are unable to discriminate fully between related opiate peptides. To date all reported RIAs for Met-enkephalin cross-react with Leu-enkephalin and the larger endorphins. Thus to establish the presence or absence of Met-enkephalin in plasma we needed (1) to develop a sensitive RIA which did not cross-react with Leu-enkephalin and β-endorphin (the latter is known to be present in human CSF[14] and plasma of normal subjects[15]); (2) to develop a system for extracting Met-enkephalin from plasma in order to concentrate it and to remove interference in the RIA by proteins and to minimise proteolysis; (3) to demonstrate any measured Met-enkephalin immunoreactivity is not an artefact of sample collection, extraction and the RIA system and (4) to demonstrate that any measured immunoreactivity extracted from plasma would co-elute on chromatography with standard Met-enkephalin. These aims have been achieved by the novel approach of developing a RIA to the methionine sulphoxide analogue of Met-enkephalin and oxidation of all samples by hydrogen peroxide before assay and after extraction by octadecasilyl-silica (ODS-silica). The RIA and extraction methodology will be described in detail elsewhere[16].

We have observed that the C-terminal methionine residue of Met-enkephalin was unstable and underwent variable spontaneous oxidation to methionine sulphoxide (1–10%) during storage, extraction and assay procedures. No reported RIAs would detect this and yet, because of the oxidative conditions of iodination ^{125}I-labelled Met-enkephalin is likely to have a C-terminal methionine sulphoxide. We decided to raise high-titre antibodies to the methionine sulphoxide analogue of enkephalin (Met-O-enkephalin) in rabbits using as the antigen Met-O-enkephalin coupled via the N-terminus to bovine thyroglobulin by the glutaraldehyde method. This antibody does not cross-react with 1,000 molar excess of Leu-enkephalin, or with purified human β-lipotrophin (β-LPH) or β-endorphin (Fig. 1) before or after their oxidation by hydrogen peroxide. Purified human LPH 61–79 cross-reacts minimally (0.02%) after oxidation. There is minimal cross-reaction with synthetic α-endorphin (0.025%) and β-endorphin (0.25%) after oxidation. We

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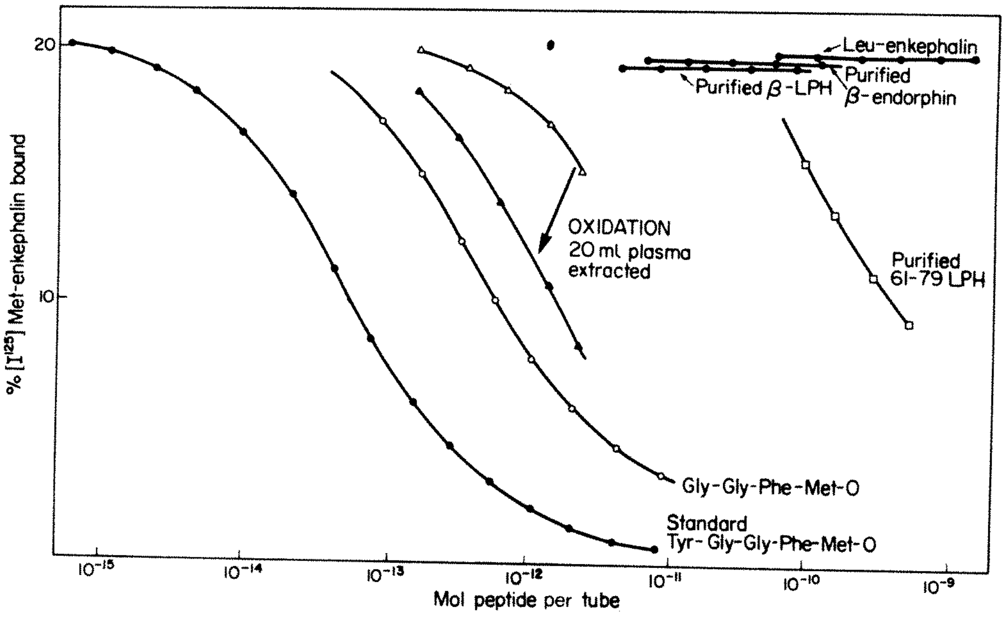

Fig. 1 Standard curve for Met-O-enkephalin and cross-reactivity studies. 50 μl of antiserum (1 in 9,000 final dilution for antibody E2) was added to 200 μl buffer containing doubling dilutions of standard Met-O-enkephalin and incubated for 24 h at 4 °C. Mono-iodinated Met-enkephalin (10 pg in 50 μl diluent buffer) was added with further incubation for 24 h before separation of bound from free [I^{125}] Met-enkephalin by double antibody precipitation. The cross-reactivities of Leu-enkephalin, purified human β-LPH, LPH 61–79 and β-endorphin and Gly-Gly-Phe-Met are shown after oxidation by 100 nmol hydrogen peroxide. A sample of 20 ml of plasma from a normal subject was extracted in duplicate on ODS-silica (as described in legend to Fig. 2) and the 80% methanol/1% formic eluates treated with (▲) or without (△) 100 nmol hydrogen peroxide before drying down by air, reconstitution and assay for Met-enkephalin.

have found that 60% and 90% respectively of their cross-reaction is due to the contamination of these synthetic preparations by Met-enkephalin. Using this antibody and mono-iodinated Met-enkephalin tracer highly purified on ODS-silica (10-μm particles) the RIA has a sensitivity of 0.01 pmol ml^{-1}. Our extraction system uses coarse ODS-silica (35–70 μm) in 1% formic acid. It allows concentration of 1–25 ml of plasma, is able to exclude the nonspecific depression of binding of tracer by plasma proteins and minimises proteolytic activity by virtue of being carried out at *p*H 1.5. This system also maximises the recovery of Met-enkephalin since the larger peptides β-LPH and β-endorphin elute poorly from coarse ODS-silica with 80% methanol/1% formic acid. When Met-O-enkephalin is added to 1 ml plasma and extracted there is 80–90% recovery. The poorer recovery of Met-enkephalin (20–30%) after extraction is related to losses during the oxidation by hydrogen peroxide. Our extraction and assay system has the inbuilt control of increased immunoreactivity diluting in parallel after treatment with hydrogen peroxide if the material is Met-enkephalin.

With this extraction and assay system and suitable collection of plasma samples to minimise degradation, 4 ml plasma samples from 20 normal subjects taken at 9 a.m. yielded Met-enkephalin immunoreactivity of 14–140 pg ml^{-1}. Repeated assay of samples from the same plasma pool gave an intra-assay variation of 10.2% ($n = 8$) at a mean value of 22 pg ml^{-1}. When 20 ml of plasma was extracted and treated with or without H_2O_2 an increase in immunoreactivity with H_2O_2 was seen (Fig. 1) suggesting this material is Met-enkephalin. The assay does have a slight cross-reaction (0.5%) with the tetrapeptide Gly-Gly-Phe-Met and 10% with Gly-Gly-Phe-Met-O. There was no cross-reaction however with smaller fragments of Met-enke-

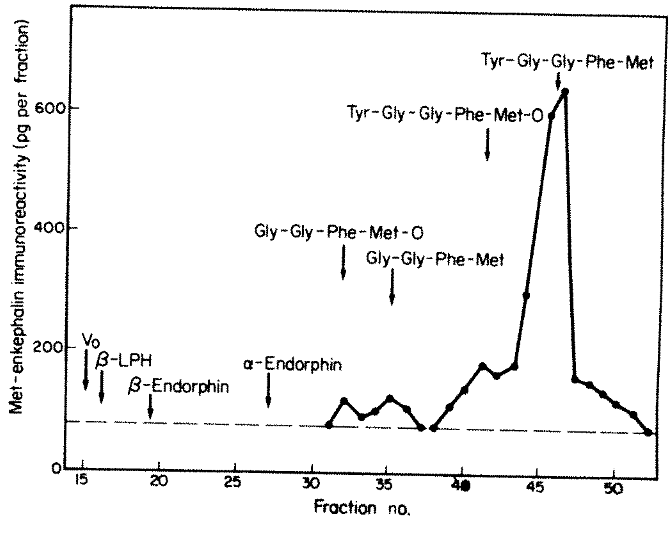

Fig. 2 A 100 × 1 cm BioGel P4 (200–400 mesh) column was equilibrated with 1% formic acid and pumped at room temperature at 3 ml h^{-1}. The column was presaturated with human serum albumin 3 mg (HSA, Blood Products Laboratory, Elstree) and calibrated initially for standard synthetic Met-enkephalin (Bachem) and then synthetic Gly-Gly-Phe-Met (Reckitt and Colman). The amino acid composition of both peptides was checked by amino acid analysis after acid hydrolysis. Fractions of 2 ml were collected and assayed for Met-O-enkephalin after oxidation by 100 nM H_2O_2, drying down with air and reconstitution with 0.05 M sodium phosphate *p*H 7.4 containing 0.25% HSA and 0.1% 2-mercaptoethanol and phenol red as indicator. V_0 indicates the void volume as shown by the HSA marker. Purified β-LPH, β-endorphin and synthetic α-endorphin were loaded and elution pattern determined by RIA of N- and C-terminal β-LPH[14,21] and α-endorphin. Samples of 160 ml of whole blood were collected from normal subjects at 9 a.m. into 10 ml lithium heparin tubes containing 10,000 KIU (Kallikrein inactivator unit) aprotinin (Trasylol) and centrifuged immediately at 4 °C. Pooled plasma (80 ml) was acidified to *p*H 1.5 by the addition of 150 μl glycine-HCl (1.6% glycine in 1M HCl) per ml of plasma and formic acid to final concentration of 1%. Plasma was extracted and concentrated in 8-ml batches on 1.5 × 0.5-cm columns containing 35–70 μm ODS-silica[16]. Acidified plasma was loaded in 800 μl aliquots followed by 800 μl of a solution of 0.9% NaCl and 1% formic acid containing 1.5 ml glycine-HCl per 100 ml. After loading 8 ml of acidified plasma a final wash with 800 μl 1% formic acid was followed by 1.6 ml of 80% methanol/1% formic acid which eluted the adsorbed peptides into a round-bottomed plastic tube containing 100 μg polypeptide (Sigma Polypep P5115) and 10 mg mannitol. Each 1,600 μl eluate was evaporated to dryness by oxygen-free nitrogen at 50 °C and each reconstituted in 200 μl 1% formic acid. After centrifugation at 2,000*g* the 2 ml of combined supernatant was loaded on BioGel P4 column after addition of 3 mg HSA. The column was eluted with 1% formic acid and pumped at 3 ml h^{-1} at room temperature and 2 ml fractions collected. H_2O_2 100 nmol was added to each fraction and left for 12 h before evaporation by compressed air in a sandbath at 50 °C and assay in duplicate for Met-O-enkephalin after reconstitution in assay buffer. If required *p*H was corrected to 7.4 by dropwise addition of 1.1M $NaHCO_3$.

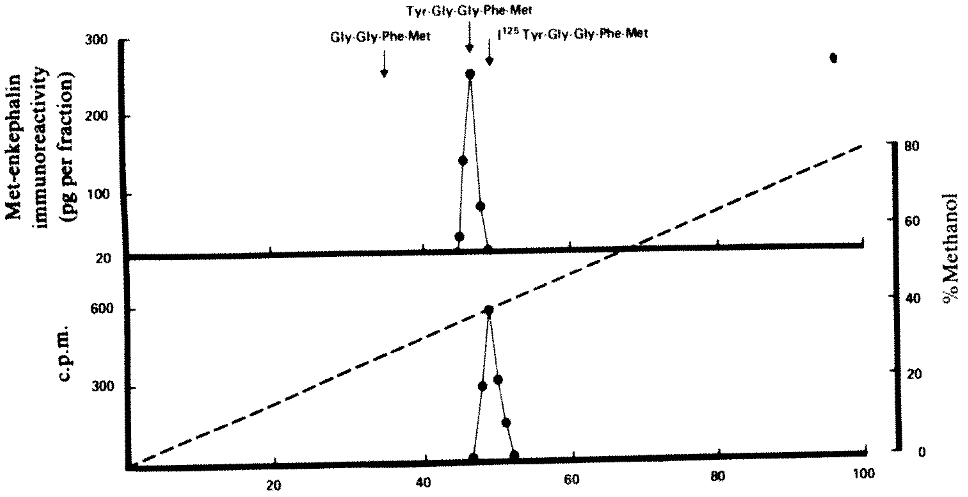

Fig. 3 High-pressure liquid chromatography (Pye Unicam LCXP) of the main plasma Met-enkephalin peak from BioGel P4 chromatography. The column (50 mm × 4 mm, Spherisorb ODS 10 μm) was developed with a linear gradient (– – – –) from 1% trifluoroacetic acid to 80% methanol/1% trifluoroacetic acid at 2 ml min^{-1} collecting 0.2 min fractions. All fractions were assayed for Met-enkephalin as described in the legend to Fig. 2. The limit of detection was 20 pg per fraction. Immunoreactive enkephalin eluted in the expected position of synthetic Met-enkephalin (upper chromatogram) two fractions earlier than 2 pg of monoradioiodinated Met-enkephalin[16] (lower chromatogram) added as internal marker. The arrows indicate the elution positions of synthetic Gly-Gly-Phe-Met, Tyr-Gly-Gly-Phe-Met and monoradioiodinated Tyr-Gly-Gly-Phe-Met appearing at fractions 35, 47 and 49 respectively in this system.

phalin[16]. Thus we could be detecting the inactive Gly-Gly-Phe-Met which, from degradation studies, might be the expected main circulating form of Met-enkephalin[12]. To examine this possibility we subjected plasma concentrated on ODS-silica to chromatography on BioGel P4. We initially calibrated the column for synthetic Met-enkephalin, Gly-Gly-Phe-Met, β-LPH, β-endorphin and α-endorphin as shown in Fig. 2. Met-enkephalin elutes in fraction 45 with a smaller peak at fraction 42 corresponding to Met-O-enkephalin generated by spontaneous oxidation. The elution of Met-enkephalin is retarded on BioGel P4 because of its hydrophobicity. This would explain the earlier elution position of Met-O-enkephalin which is less hydrophobic. Gly-Gly-Phe-Met similarly elutes into two peaks of oxidised and native peptide but well separated from the peaks of Met-enkephalin.

Figure 2 shows the chromatography of 80 ml of ODS-extracted plasma on BioGel P4. The elution profile shows the main peak of immunoreactivity at fraction 45 co-eluting with the known position of Met-enkephalin and a smaller peak at fraction 35 corresponding to the position of Gly-Gly-Phe-Met. There are also small peaks corresponding to Met-O-enkephalin and Gly-Gly-Phe-Met-O; these might be expected from spontaneous oxidation during extraction and chromatography. Further evidence for the identity of the main peak from the BioGel P4 column was obtained from reverse phase HPLC (Fig. 3).

These data suggest that the bulk of immunoreactivity in plasma after extraction in conditions which inhibit proteolytic degradation is Met-enkephalin (85%), a small amount (15%) may be due to the tetrapeptide Gly-Gly-Phe-Met which has lower cross-reactivity. Since we can demonstrate that the recovery after ODS extraction of Gly-Gly-Phe-Met and Met-enkephalin added to acidified plasma is equal we cannot have preferentially extracted either peptide before BioGel P4 chromatography.

When human plasma containing exogenous or endogenous β-LPH or β-endorphin was incubated for several hours at room temperature[16] we failed to detect an increase in Met-enkephalin immunoreactivity suggesting that the Met-enkephalin we detect in plasma is unlikely to be an artefact of our extraction system formed by degradation of plasma β-LPH or β-endorphin.

Dexamethasone treatment (2 mg at midnight) of seven normal subjects suppressed plasma immunoreactive ACTH and β-LPH to undetectable levels but did not alter the circulating Met-enkephalin levels significantly ($P > 0.2$). Samples collected from two normal subjects at 9 a.m. and midnight did not show a nyctohemeral rhythm in Met-enkephalin secretion as is observed for ACTH and β-LPH[17]. These data suggest that the Met-enkephalin-like immunoreactivity in plasma is independent of circulating ACTH, β-LPH and β-endorphin, which have been shown to be secreted in parallel from the pituitary gland in response to different stimuli[18]. To determine the source of circulating Met-enkephalin we measured samples collected at selective venous catheterisation of a patient undergoing investigation for hirsuties. Although the level of Met-enkephalin in venous blood taken from the left internal jugular (55 pg ml^{-1}) was the same as the peripheral level (53 pg ml^{-1}), simultaneous peripheral and left adrenal vein samples showed twice the concentration in the adrenal effluent (116 pg ml^{-1}). These results suggest that Met-enkephalin is secreted into the circulation from the adrenal glands. Immunoreactive Met-enkephalin has been detected in whole rat adrenal by RIA[19]. Furthermore immunocytochemical studies of the adrenal show the Met-enkephalin located mainly in the adrenal medulla[20]. Fresh human adrenal tissue was carefully dissected into medulla and cortex and each homogenised in boiling 0.1 m HCl. After the addition of formic acid to the supernatant to final concentration of 1%, Met-enkephalin was measured after coarse ODS extraction, using the method as described for plasma. The majority of the immunoreactive Met-enkephalin was located in the medulla (0.8 μg per g) with small but detectable amounts in the cortex (0.02 μg per g).

We conclude that Met-enkephalin circulates as the intact pentapeptide in human plasma and is possibly secreted by the adrenal medulla. Plasma levels of Met-enkephalin are unrelated to the levels of endogenous ACTH, β-LPH and β-endorphin which adds further support to the argument that Met-enkephalin belongs to a separate neuropeptide system with its own precursor.

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GABA and picrotoxinin receptors in clonal nerve cells

C. Napias & R. W. Olsen

Division of Biomedical Sciences and Department of Biochemistry,
University of California, Riverside, California 92521

D. Schubert

Department of Neurobiology, The Salk Institute for Biological Studies,
PO Box 1809, San Diego, California 92112

γ-Aminobutyric acid (GABA) is a neurotransmitter which seems to mediate inhibitory synaptic transmission in many regions of the central nervous system (CNS) of vertebrates and invertebrates[1]. GABA has been shown to act by increasing membrane chloride permeability in invertebrate muscle[2,3], and a similar mechanism has been suggested for the vertebrate CNS[4]. The response to GABA is inhibited noncompetitively by the convulsant picrotoxinin[1–5]. Binding sites for radioactive GABA[6–8] and picrotoxinin[9–11] (the active component of picrotoxin) have been described in homogenates of invertebrate muscle and mammalian CNS; these binding sites show many properties expected of postsynaptic membrane macromolecules involved in the receptor–ionophore function of GABA. Nevertheless, unambiguous correlation between biochemical assays and *in vivo* function is difficult to obtain, especially in heterogeneous tissue such as mammalian CNS. One approach to this problem involves cloned cell lines of neuronal origin which retain in culture the differentiated function to be studied (see, for example, ref. 12). Certain cell lines cloned from rat[13] and mouse[14] nervous system possess neuronal properties such as electrical excitability, neurotransmitter synthetic enzymes[12], neurotransmitter uptake[15,16], binding of defined ligands to their appropriate neurotransmitter receptors[12,17,18], and receptor-coupled responses to neurotransmitters[17,19,20]. Several cell lines of apparent glial origin have also been described[13,21,22]. To determine whether binding sites for GABA and picrotoxinin are localised to nerve cells, a variety of clonal cell lines were assayed for their ability to bind those ligands. We report here the presence of GABA and picrotoxinin binding sites with receptor-like properties in several clonal nerve cell lines obtained from the rat CNS, and the absence of such binding sites in many other cell lines of non-neuronal origin.

Table 1 shows that GABA receptor-like binding and α-dihydropicrotoxinin (DHP) binding were present in membranes from all five rat CNS nerve lines examined, whereas no binding was seen with the non-neuronal lines. GABA binding was measured as that fraction of radioactive GABA pelleted with the membranes that was displaceable by 10^{-5}M muscimol, in sodium-free buffer, using membranes thoroughly washed with buffer and repeatedly frozen and thawed. Extensive washing is required to remove endogenous inhibitors of GABA binding that were seen in the extracts of these cells (data not shown), as previously observed in brain[8]. Binding of GABA in such conditions has been well demonstrated to reflect receptor-like specificity[6–8,23].

Binding of ^{3}H-GABA and ^{3}H-DHP to one of the nerve cell lines, B35, was studied in detail for comparison with the well characterised binding of these ligands to rat brain homogenates. Figure 1 shows a Scatchard plot of sodium-independent GABA-displaceable binding against GABA concentration for B35 membranes. An apparent single class of affinity ($K_D = 22$ nM) was observed, with a total capacity of sites (maximal binding at saturating ligand concentration) of 250 pmol per g protein. This quantity of sites is roughly equal to the total number of sites in some of the less dense regions in CNS[23–25]. Assuming that levels of binding activity can be compared in one set of constant assay conditions, the level in the other four lines tested was slightly lower (Table 1). The absolute amount of specific GABA binding did, however, vary between different preparations of the same cell line. This is probably due to slight differences in growth state and is under investigation. The GABA binding affinity to B35 membranes was equal to that of one of the two classes of sites in mammalian brain which have K_D values of 20 and 180 nM (refs 8, 25). No low-affinity GABA binding corresponding to the brain site having a K_D of 180 nM could be detected in B35 cell membranes in three experiments. Considering only the high-affinity GABA binding sites, the number of sites in B35 cells was about the same as that for this class of sites in high-density brain regions[25].

Figure 1 (inset) shows that this high-affinity sodium-independent GABA binding to B35 cells was displaced by GABA analogues with the specificity expected of receptor sites. The receptor-specific drugs muscimol ($IC_{50} = 50$ nM in B35, 25 ± 15 nM in brain)[7] and 3-aminopropane sulphonate ($IC_{50} =$ 500 nM in B35, 85 ± 15 nM in brain)[7] were good inhibitors, with concentrations $>10^{-5}$ M displacing the same amount of ^{3}H-GABA as did 10^{-4}M nonradioactive GABA. However, the transport-specific analogue 2,4-diaminobutyrate was not active ($IC_{50} > 10^{-4}$M in both brain and cell line B35). Bicuculline was a modest inhibitor of GABA binding in B35 and picrotoxinin had no effect up to 10^{-4}M.

Table 1 Specific GABA and DHP binding to membranes from cultured cell lines

Cell line	Cell type	Ref.	DHP bound (fmol per mg protein)	GABA bound (fmol per mg protein)
B35	CNS nerve	13	850 ± 100	130 ± 20
B50	CNS nerve	13	513 ± 90	77 ± 10
B65	CNS nerve	13	378 ± 80	25 ± 3
B103	CNS nerve	13	394 ± 80	48 ± 4
B104	CNS nerve	13	252 ± 50	32 ± 4
B9	CNS glial	13	NS	NS
B12	CNS glial	13	NS	NS
B15	CNS glial	13	NS	NS
B82	CNS glial	13	NS	NS
PC12	Phaeochromocytoma exponentially dividing	27	NS	NS
PC12	Stationary phase	27	NS	NS
L6	Skeletal myoblast	28	NS	NS
S194/5	Plasmacytoma	29	NS	NS

The clonal cell lines were grown as described in the references indicated. With the exception of PC12, which was collected at both the exponential (1×10^6 cells per 100-mm diameter dish) and stationary phase of growth, all cells were collected from early stationary phase cultures, 2 days after cessation of net cell division, at a density of $4–6 \times 10^6$ cells per 100-mm diameter dish. The cells were greater than 95% viable as measured by the uptake of fluorescein dibutyrate. During preparation, the cells were washed twice in phosphate-buffered saline (150 mM NaCl, 10 mM Na phosphate, *p*H 7.3, 2 mM $CaCl_2$, 0.5 mM $MgCl_2$), frozen as a pellet (0.2–1 g) on dry ice and stored at −85°C. These were thawed in 5 ml distilled H_2O and sonicated twice for 30 s (MSE sonifier, maximum setting) at 0–4 °C separated by a 30 s cooling break. The suspension was then homogenised by hand at 0 °C with five passes of a glass–glass Dounce homogeniser, diluted to 9 ml and centrifuged for 30 min at 200,000*g* [Beckman rotor 65 (49,000 r.p.m.)]. For DHP binding studies, the pellet was rehomogenised at 0–4°C with a Potter–Elvehjem homogenising tube and motor-driven pestle, five passes at 600 r.p.m., in 0.2 M NaCl, 10 mM Na phosphate, *p*H 7.0, recentrifuged as above and rehomogenised as before in the same buffer for assay[10]. For GABA binding assays[8], the first pellet obtained after homogenisation was rehomogenised in 10 ml of 50 mM Tris buffered to *p*H 7.1 at 4 °C with citric acid (sodium-free), and frozen at least 15 h. On the day of assay, this homogenate was thawed, pelleted as before, rehomogenised in the same buffer and repelleted, then rehomogenised in the same buffer for assay. The washing procedure is suitable for the removal of endogenous inhibitors of GABA binding in brain memranes[8]; further washing of the membranes obtained from the cells did not alter GABA binding. The protein concentration was adjusted to 0.5 mg in 1 ml assay volume. Binding of both ligands was determined by centrifugation at 48,000*g* (20,000 r.p.m., Sorval rotor SM24) following incubation in triplicate at 0–4 °C for 15 min, at which time equilibrium was obtained[8,9]. ^{3}H-GABA (Amersham, 54 Ci mmol^{-1}) was included at 9.3 nM, with background (nondisplaceable radioactivity pelleted) estimated with 10^{-5}M muscimol. The nondisplaceable background was approximately 4,000 c.p.m. in each case, and the specific binding varied over 4,000–12,000 c.p.m. ^{3}H-DHP (Amersham, 26 Ci mmol^{-1}) was 63 nM, with background estimated by 10^{-4}M nonradioactive DHP. Background was generally 20,000 c.p.m., and specific binding varied over 3,000–10,000 c.p.m. After pelleting, the tubes were superficially rinsed twice, not disturbing the pellets, with 5 ml assay buffer, and the pellets were dissolved overnight at 22 °C in 0.1 ml Soluene-350 (Packard). Radioactivity was measured in a Beckman LS-100C scintillation counter with an efficiency of 40%, using a 0.5% diphenyloxazole PPO in toluene. Each value is the mean of two experiments in triplicate, ± standard deviation. NS, not significant at 90% confidence level (<100 fmol DHP bound per mg protein or <5 fmol GABA bound per mg protein at the concentrations of ligand used).

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Table 2 Drug inhibition of DHP binding to B35 clonal nerve cells

Displacing agent (M)	Per cent inhibition of specific binding				
	DHP	Picro-toxinin	DMBB	Mepho-barbital	IPTBO
10^{-7}	5 ± 5	20 ± 5	—	—	—
5×10^{-7}	42 ± 10	—	—	—	—
10^{-6}	—	—	—	—	—
2×10^{-6}	84 ± 20	—	—	—	—
10^{-5}	93 ± 20	87 ± 20	106 ± 20	—	—
10^{-4}	100 ± 20	—	—	76 ± 20	66 ± 20

Membranes were prepared from cells grown as described in Table 1, and assayed for ^{3}H-DHP binding[10] in the presence of various concentrations of the drugs indicated. DHP, nonradioactive α-dihydropicrotoxinin; DMBB, 1,3-dimethylbutylbarbiturate; IPTBO, isopropylbicyclophosphate. ^{3}H-DHP was 63 nM, 26 ± 5 Ci mmol^{-1}; protein 0.5 mg in 1 ml assay volume. The controls gave roughly 8,000 c.p.m. bound and a background of 20,000 c.p.m. Each value is the mean of two experiments done in triplicates.

These specificity properties are similar but not identical to the properties described for rat brain[7]. The small differences may reflect the presence of fewer classes of binding site populations in the B35 cells than in brain, and/or the receptor properties in the cells may be different because of a location in membranes with incompletely differentiated synaptic characteristics. Slight modifications unique to the cell line or its growth conditions are also possible.

The DHP binding to B35 cells was also further characterised (Table 2). Properties similar to those found for the binding sites in brain were observed, with 50% displacement by about 10^{-6}M nonradioactive DHP (brain $K_D = 1.2\times10^{-6}$ M)[10], and by 10^{-6} M picrotoxinin (brain $IC_{50} = 0.4\times10^{-6}$ M)[10], and 100% displacement by 10^{-5} M 1,3-dimethylbutylbarbiturate (brain $IC_{50} = 5\times10^{-8}$ M)[11], 76% displacement by 10^{-4} M mephobarbital (brain $IC_{50} = 5\times10^{-6}$ M)[11] and 66% displacement by 10^{-4} M isopropylbicylophosphate (brain $IC_{50} = 8\times10^{-6}$ M)[10].

Thus, both GABA and DHP binding sites in B35 membranes had properties similar to those characterised in brain. The specific GABA receptor and DHP binding sites were observed only in cell lines of CNS origin which have neuronal properties, and were absent from several other types of cell. The above results open the way for further studies of this neurotransmitter using the clonal cell cultures. This system should facilitate the study of several questions regarding GABA function, such as the regulation of chloride permeability, the heterogeneity in binding sites, the function of endogenous inhibitors[8] and the interactions with barbiturates[11] and benzodiazepines[26].

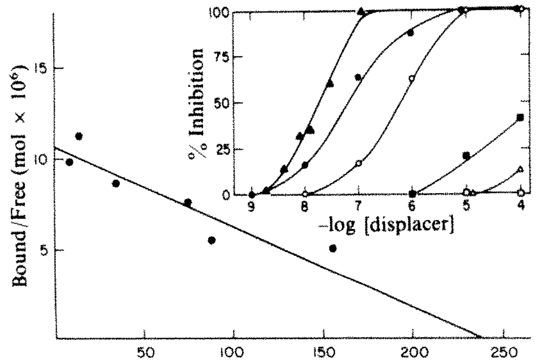

Fig. 1 Scatchard plot of GABA binding to B35 clonal nerve cells. Membranes were prepared and assayed[8] as described in Table 1 legend. Protein was 0.7 mg in 1 ml assay volume, ^{3}H-GABA (54 Ci mmol^{-1}) was varied over 0.1–10 nM, and nonradioactive GABA over 10 nM–2μM, with 10^{-4} M nonradioactive GABA used to estimate nondisplaceable background. Each point is the mean of triplicates and the experiment is typical of three. The ratio of specific binding to background varied from 3.0 (low concentrations) to 0.4 (high concentrations), with standard deviations of ≤4% in the raw data and ≤10% in the specific binding. Inset: receptor specificity of GABA binding to B35 neuroblastoma cells. ^{3}H-GABA was 9.3 nM. Control binding was about 6,000 c.p.m. with background of 4,000 c.p.m. Binding was measured in the presence of various concentrations of displacing agent. Each point is the mean of triplicates and typical of two experiments. ●, Muscimol; ○, 3-aminopropane sulphonate; △, 2,4-diaminobutyrate; ■, bicuculline; □, picrotoxinin; ▲, GABA.

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Quantitative microlocation of lithium in the brain by a (n, α) nuclear reaction

M. Thellier, J. C. Wissocq & C. Heurteaux

Laboratoire des Échanges Cellulaires, Centre de Recherches en
Biochimie et Physiologie Cellulaire, Faculté des Sciences et
Techniques, 76130 Mont-Saint-Aignan, France

Although lithium has long been used in psychiatric practice[1–4], its exact mechanism of action remains largely unknown. The reason is mainly methodological. Lithium has no radioisotope, and it is too light to be detectable by electron-probe microanalysis. To study the distribution of lithium in the brain, most authors[5] have proceeded by dissecting the brain into small fragments and determining the mean Li content of each fragment by photometric methods. However, the resolving power by this method has remained poor. Our group[6] and others[7] have recently described the possibility of using the stable isotopes of lithium as tracers for estimating unidirectional fluxes. To study Li-location, there is the theoretical possibility of using the specific nuclear reaction ^{6}Li(n, α)^{3}H. The method cannot be used as a conventional radioactivation because the induced ^{3}H has negligible radioactivity compared with the many other radioisotopes also induced in a biological sample bombarded by neutrons. The detection then consists of laying sections of the ^{6}Li-containing samples in contact with a convenient detector, and irradiating the whole arrangement with neutrons; the distribution of lithium in the sample is revealed by the tracks of the α and ^{3}H particles in the detector. Some authors have already attempted to use such methods for various biological applications[8–15]. By taking advantage of different independent technical advances[16–22], we can now give a precise quantitative picture of the distribution of lithium in the brain of a lithium-treated mouse.

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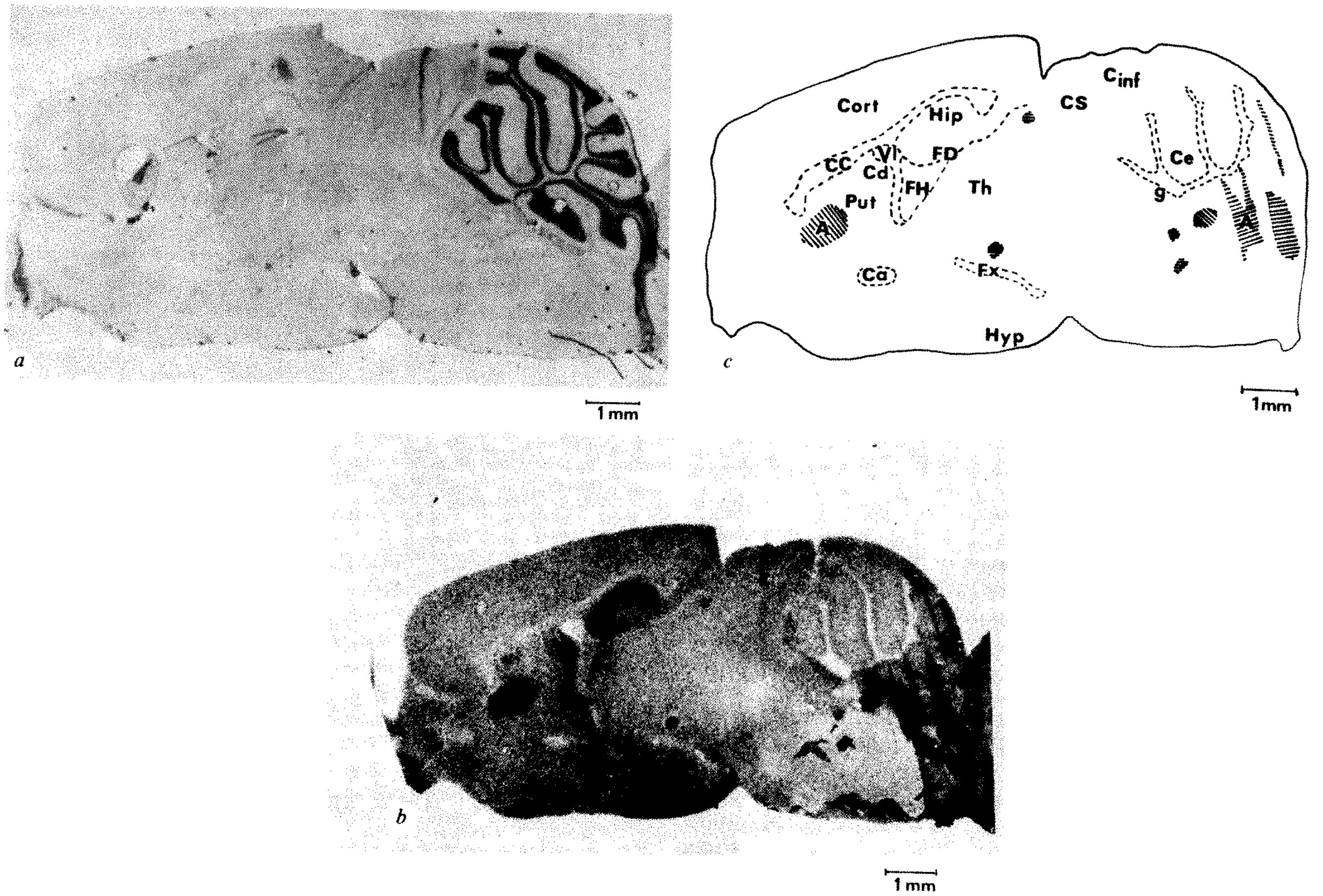

Fig. 1 Lithium distribution in a mouse brain. *a*, Histological preparation stained by Feulgen method. *b*, Photograph of the corresponding detector (each small black spot which forms the shading seen corresponds to a nuclear reaction on lithium). *c*, Schematic representation of the brain microanatomy at the level of the section. The mice had been treated with a daily dose of 12 mequiv. per kg lithium for 3 days, with the last dose 6 h before the experiment. Lithium was given as LiCl in the form of ^{6}Li-enriched isotope 96%. The mice were decapitated, and each brain was immediately removed and immersed in liquid isopentane cooled by liquid nitrogen. Sagittal sections (10 μm thick) of the frozen brains were made with a Jung microtome placed in a WKF chamber cooled to −35 °C. The sections were deposited on quartz supports, lyophilised, tightly pressed against Kodak LR 115 cellulose nitrate detectors, and neutron irradiated. A, Mechanical defects on the detector (dashed areas on *c*); Ca, Commissura anterior; CC, corpus callosum; Cd, nucleus caudatus; Ce, cerebellum; Cinf, inferior colliculus; Cort, neocrotex; CS, superior colliculus; FD, fascia dentata; FH, fimbria hippocampi; Fx, columna fornicis; Hip, hippocampus; Hyp, hypothalamus; g, grana; Put, putamen; Th, thalamus; Vl, ventriculus lateralis.

Figure 1 shows our results. The detectors show a certain number of defects seen as dark spots (especially at the level of the midbrain) or scratches (on the cerebellum); however, the different brain substructures are easily recognisable. The hippocampus, fascia dentata, nucleus caudatus, hypothalamus and neocortex are the substructures most heavily loaded with lithium. The cerebellum is also rich in lithium, except for the white matter inside the lobes. At the level of the putamen, careful observation shows lithium-rich striae corresponding approximately to the striae of grey matter. The fimbria hippocampi, superior colliculus and inferior colliculus have moderate lithium concentrations, whereas the thalamus has a rather small lithium content. Even smaller lithium concentrations are found in the corpus callosum, commissura anterior, columna fornicis and the cavity of the ventriculus lateralis. Clearly, the areas which achieve the most integrated operations for the control of motricity, behaviour and emotional attitudes are also those with the highest lithium concentrations. Therefore, in future work, it might be interesting to compare the relative distribution of lithium in the brain of control animals with that in the brain of animals subjected to various stresses, drugs or surgical operations known to affect sensory representation, motricity or behaviour. The resolving power of our method is already good enough to attempt such studies at the histological level. Moreover, as the theoretical limit of the resolving power is estimated[23] to be about 20 Å, further progress in the technology of track detection should eventually allow us to extend the use of the method to the cytological level.

The accumulation of lithium by the hypothalamus can be discussed in relation to the neuroendocrine properties of that area. We have indicated previously[20] that another endocrine gland, the hypophysis, also exhibited a high lithium concentration after treatment of the animals with lithium. Moreover, recent data obtained with Vaudry and Tonon (unpublished) indicate that the release of α-melanocyte-stimulating hormone by frog hypophysis is severely inhibited by lithium. This interference with neuroendocrine regulation might have significance in the whole-body effects of lithium.

Figures 2 and 3 are enlarged from Fig. 1, at the level of the cerebellum and the hippocampus. Although there is not a direct law of proportionality, the areas which accumulate much lithium are most generally those with the largest densities of neurone bodies. The reason is perhaps that the distribution of lithium is determined by cell water content at the various areas of the brain; thus, the nerve trunks, being highly lipid, would fail to reveal much lithium uptake. Whatever the reason, this result at least indicates that lithium effects on the brain might originate from the perturbation of the metabolism of some nerve cells, rather than from a direct effect on the process of nervous conduction. For instance, lithium could interact with the biosynthesis of some important proteins in the nervous system. Indeed, in a variety of other cases (sea-urchin embryology[24], aging processes[25,26], growth [27] and bud precedence[28] in plants), lithium affects the biosynthesis of some specific proteins, probably at the level of transcription[24], without necessarily modifying the bulk of the other protein biosynthesis[26,27].

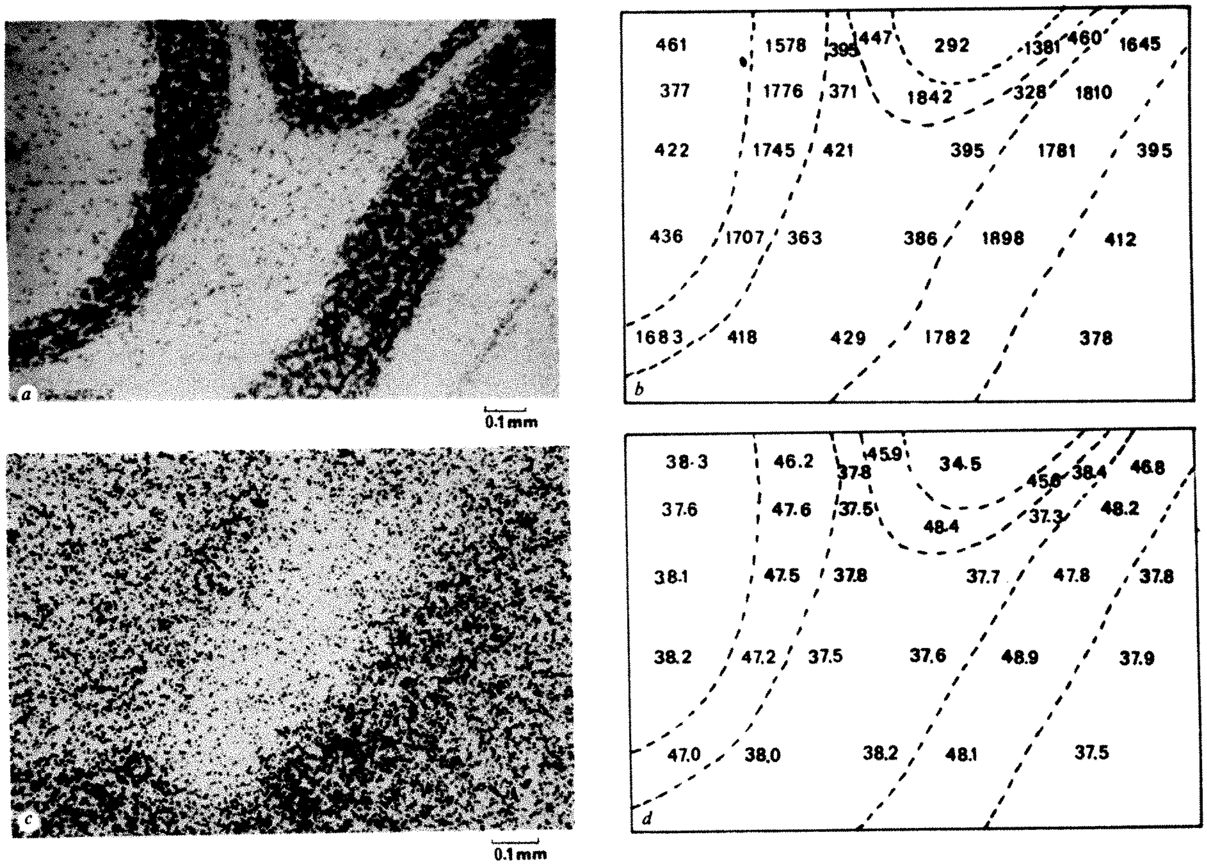

Fig. 2 Enlargement of Fig. 1, at the level of the cerebellum. *a*, Histological preparation; *b*, corresponding estimation of the local densities of cellular nuclei (nuclei per μg); *c*, photograph of the corresponding detector; *d*, estimation of the corresponding local concentrations of lithium (pequiv. per μg).

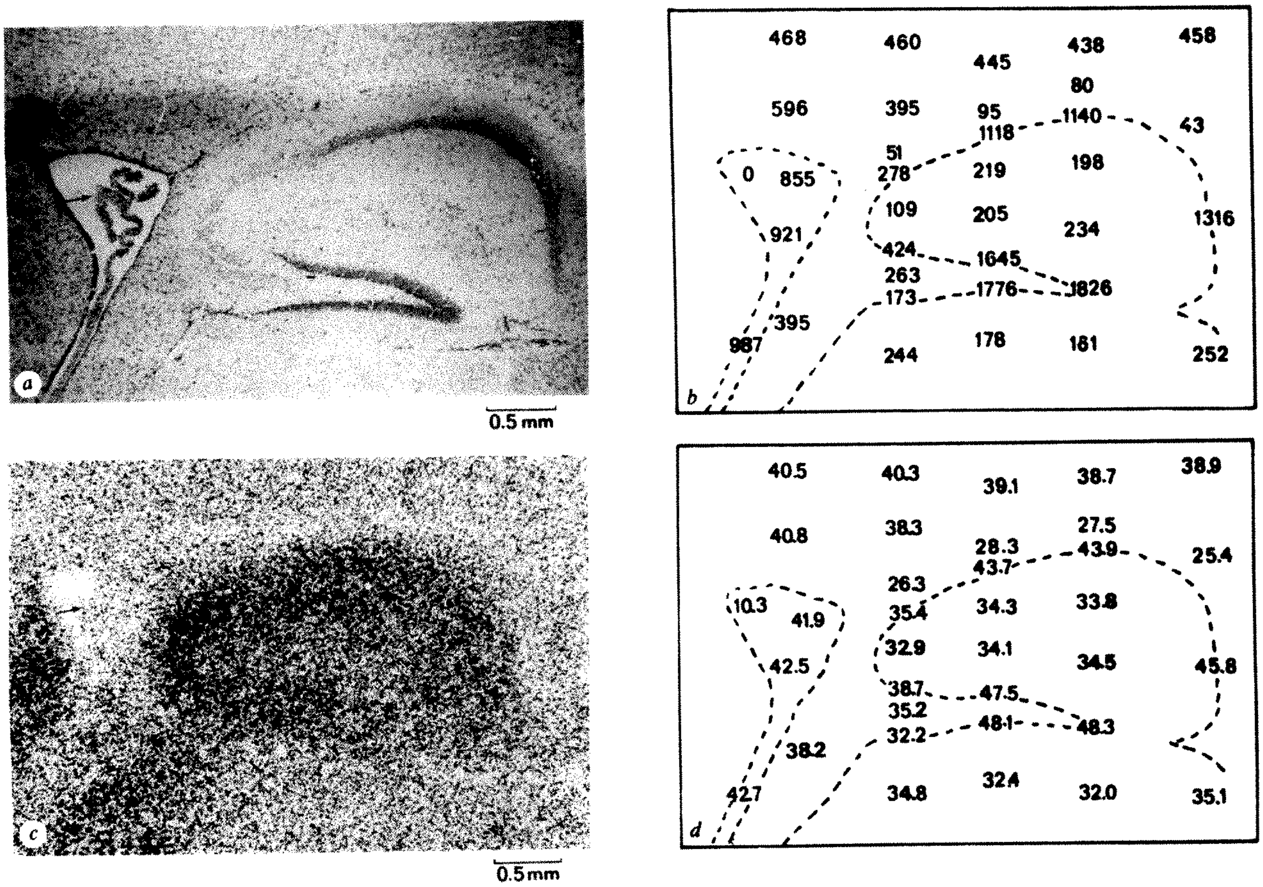

Fig. 3 Enlargement of Fig. 1, at the level of the hippocampus. Same significance of *a*–*d* as in Fig. 2. To appreciate the resolving power of the method, note the correspondence between the histological structures on the section and the distribution of tracks on the detector, especially at the level of the black arrow.

The nuclear track technique can be extended to study the distribution of elements other than lithium. Our group and others have already begun to use it for studying the distribution of boron in plants[29,30] or in semiconducting alloys[31], and the labelling of oxygen[32] by stable isotope ^{17}O for the location of catecholamines in the rat brain[33].

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Parasite-induced 'nonspecific' IgE does not protect against allergic reactions

Ellen Jarrett & Susan Mackenzie

Department of Veterinary Parasitology,
University of Glasgow Veterinary School, Bearsden, Glasgow, UK

Hans Bennich

Biomedical Center, Uppsala University, Uppsala, Sweden

High levels of immunoglobulin E (IgE) are produced in animals, including man, infected with helminth parasites[1,2]. Such infection results not only in the production of parasite-specific IgE but also, through polyclonal stimulation of IgE-B cells, in the production of a large excess of parasitic nonspecific IgE[3]. IgE antibodies mediate allergic reactions by binding to receptors on the plasma membrane of mast cells, which are then said to be sensitised; subsequent attachment of antigen to the membrane-bound antibody causes cross-linking of the receptors leading to the release of histamine and other active substances[4]. Mast cells can be passively sensitised both *in vivo* and *in vitro* and passive sensitisation with a specific IgE antibody may be inhibited by prior saturation of IgE receptors with an excess of 'nonspecific' IgE[5,6]. In this way, helminth-infected animals become refractory to passive sensitisation, as do animals bearing IgE myelomas, because of the pre-emption of mast-cell receptors by endogenous IgE[7,8]. These findings gave rise to two related ideas: first, that it might be possible to ameliorate allergic diseases by passively administering, or by actively stimulating the production of a large amount of 'irrelevant' IgE; and second, that a high parasite-induced IgE level might protect against unrelated allergies by means of competition for mast cell receptors[6,7,9]. However, searches for epidemiological evidence of such a protective effect have had conflicting results[10–21]. We have examined this question using egg-albumin (EA)-hypersensitive rats infected with *Nippostrongylus brasiliensis* and now show that 'nonspecific' IgE induced by helminth infection, although having the capacity to block passive sensitisation, does not inhibit hypersensitivity reactions mediated by an actively produced IgE antibody.

Hooded Lister rats immunised with 10 μg of EA and the adjuvant *Bordetella pertussis*, produce IgE antibodies to EA from day 10 after immunisation. Immediate hypersensitivity reactions can then be induced in these rats by an antigen challenge; intradermal injection of EA causes an immediate wheal and flare skin reaction and intravenous injection of the antigen leads to anaphylactic shock. This state of hypersensitivity persists, as does IgE antibody production, for at least 80 days after immunisation[22]. Although, in theory, other immunological mechanisms can have a role in such allergic reactions, their contribution in the present model is likely to be minimal. IgG2a (short-term sensitising) antibodies cannot be detected in rats immunised with EA by this technique and the level of other antibodies also remains very low.

In a preliminary experiment we attempted to displace actively produced IgE from mast cells by infusing EA-immunised rats with serum from *N. brasiliensis*-infected rats having a very high IgE level: thus, rats were injected daily for 1 week with 5 ml serum containing 140 μg ml^{-1} total IgE which can be estimated to yield a serum level of 30–40 μg ml^{-1} in the recipient. The severity of allergic reactions to antigen challenge was not diminished by this treatment.

To create optimum conditions for the demonstration of an allergy-protective effect, we carried out an experiment in which EA-hypersensitive rats were infected with *N. brasiliensis* to stimulate prolonged active synthesis of high levels of irrelevant IgE. In rats infected with this parasite the level of total serum IgE rises from less than 1 μg ml^{-1} before infection to up to 400 μg ml^{-1} by the 12th day after infection[3]. The level remains elevated for many weeks and can be boosted by re-infection[22].

The design of the experiment outlined in Table 1 was based on the supposition that an observable blocking effect of nonspecific over specific IgE, in an animal actively producing both, might depend not only on the relative proportions but also on the order of production of the two. Groups of rats were therefore infected with *N. brasiliensis* 20 days before, simultaneously with or 20 days after immunisation with EA. Control groups were immunised but not infected. On various occasions after these treatments the total and antibody IgE status of the rats were assessed and susceptibility to hypersensitivity reactions was determined by antigen challenge.

Table 1 shows the results obtained on the first of these occasions, which is also representative of results obtained later in the experiment. Essentially, we were unable to induce any observable protection either in terms of skin test reactivity or susceptibility to anaphylactic shock by arranging for the occupation of mast-cell receptors with 'nonspecific' IgE whether before, during or after the synthesis of EA-specific IgE. That mast-cell receptors were indeed saturated or otherwise unavailable in the infected animals was confirmed by the finding that these animals, unlike uninfected control rats, could not be effectively sensitised for a passive cutaneous anaphylaxis (PCA)

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Table 1 Effect of nonspecific IgE induced by *N. brasiliensis* (Nb) infection on the severity of reactions to egg-albumin (EA) challenge in EA-hypersensitive rats

	Immunisation and infection*		Circulating IgE on day 32†			Hypersensitive reactions on day 34‡	
Group	Day 0	Day 20	Total IgE (μg ml^{-1})	EA-IgE PCA titre	% Rats with detectable circulating EA-IgE	Skin test reaction diameter (mm)	Anaphylaxis score (4 individual rats)
1	Nb	EA	44.6 ± 4.3	17.1	87.5	13.6 ± 0.4	4,4,4,2
2	—	EA	0.54 ± 0.06	12.9	87.5	12.2 ± 1.2	3,2,0,4
3	EA	Nb	113 ± 9.8	152.0	100.0	14.6 ± 1.0	3,3,4,4
4	EA	—	0.65 ± 0.1	21.1	87.5	12.8 ± 0.8	2,3,4,3
5	EA+Nb	—	31.5 ± 2.4	22.6 (54)	25.0 (100)	11.2 ± 2.4	2,1,0,3
6	—	—	0.48 ± 0.08	0	0	all <5	0,0,0,0

* Outbred female hooded Lister rats (Animal Suppliers) were immunised with EA by intraperitoneal injection of 10 μg EA (Sigma grade V) in 0.15 M NaCl, together with a suspension of 10^{10} heat-killed *B. pertussis*. *N. brasiliensis*, infection was by subcutaneous inoculation of 4,000 infective larvae. The method of culture of the parasite has been described previously[29]. On various occasions after the indicated treatments, eight rats from each group were bled to obtain serum for the determination of total and antibody IgE and 2 days later were challenged with EA and the resulting hypersensitivity reactions assessed. This sequence of bleeding and challenge was carried out on days 32–34, 56–58 and 78–80. The rats of group 3 were re-infected on days 43 and 63.

† Total serum IgE was estimated by a paper radioimmunosorbent test (PRIST)[30]. Results shown are the mean ±s.e. of values from eight rats. Specific IgE antibodies to EA were estimated by the passive cutaneous anaphylaxis (PCA) technique[31] as previously described[3]. Results shown are the geometric mean PCA titre of animals with a detectable response. To detect IgE antibody in the sera of group 5, the assays were subsequently repeated using the PRAST[25] technique the results of which, in units ml^{-1}, are shown in parentheses. The reason for the use of this technique is described in the text.

‡ Hypersensitivity reactions were elicited as follows. Each of four rats received four intradermal injections of 1 μg EA (in 0.05 ml 0.15 M NaCl) on the shaved back followed immediately by intravenous injection of 0.5 ml 1% Evans blue. After 20 min the rats were killed, and the skin test reactions were measured on the inner surface of the skin. The reaction diameters shown are the mean ±s.e. of the mean values from the four rats. Small reactions only (<5 mm) occurred in unsensitised rats. Anaphylaxis was induced, in an additional four rats per group, by the intravenous injection of 250 μg EA in 0.5 ml of 0.15 NaCl. Symptoms of shock occurred within seconds and were graded on a scale of severity from 1 to 4 with the latter denoting almost instantaneous death. This does of antigen caused no reaction in unsensitised rats. The amounts of antigen used were arrived at in a previous dose–effect experiment and were chosen as being sufficiently large to elicit clearly evident reactions in the majority of sensitised animals with minimal or no reactions in normal rats. The object was to operate the system at moderate antigen excess so that the occurrence and size or severity of reactions would be determined by the amount of specific IgE on the mast cell rather than by the amount of antigen injected.

reaction with IgE antibodies to keyhole-limpet haemocyanin.

Note that *N. brasiliensis* infection, in addition to stimulating nonspecific IgE, may enhance or inhibit the EA-IgE response depending on the time between immunisation with EA and infection. A pre-established EA-IgE response is briefly potentiated by the infection[22,23] as was evident in the rats of group 3 on the first occasion of testing (Table 1), but not later in the experiment. We have previously considered whether this increment in the level of EA-IgE might actually increase the degree of hypersensitivity of the animal to EA but have found that it had no noticeable effect[24]. In the context of the present experiment, even when the EA-IgE response is potentiated it still comprises a very small proportion of the total IgE[3]. In rats immunised with EA and simultaneously infected with *N. brasiliensis* (group 5), the production of EA-IgE was inhibited by comparison with rats immunised with EA alone, a phenomenon previously described by Orr and Blair[23] and presumably reflecting a form of antigenic competition. Nevertheless, most of these rats showed marked immediate hypersensitivity to EA, suggesting that EA-IgE antibodies were produced but at levels too low to be detected by the PCA technique in the face of competition by nonspecific IgE for the mast-cell receptors. The subsequent development of a radioimmunoassay for rat IgE antibodies[25] (which is not influenced by the presence of irrelevant IgE in the test serum) enabled us to retest these sera and low levels of EA-IgE were indeed detected in them all, as indicated in parentheses.

Our results suggest that as far as the actively sensitised animal is concerned, parasite-induced IgE does not have an appreciable allergy-protective effect. We now believe that the prediction of such an effect has been based on a misleading interpretation of previous passive sensitisation experiments which has failed to consider the turnover of IgE on the mast cell in active sensitisation and the relative proportions of specific and nonspecific IgE which might be expected in helminth infection.

The binding of IgE to its receptor, although of high affinity, is a reversible process (reviewed in ref. 4) and mast-cell-bound and free IgE are in dynamic equilibrium; EA-IgE therefore becomes part of a serum pool of which the constituent molecules are in constant exchange with cell-bound IgE. Therefore, assuming a similar affinity of the molecules for the receptors, EA-IgE, whether produced before, simultaneously with or after nonspecific IgE, will eventually come to be represented on the mast-cell membrane in the same relative proportion as it is found in the serum. Estimates from radioimmunoassay show that in sera from EA-immunised rats infected with *N. brasiliensis* the amount of EA-IgE produced is of the order of 1–100 ng ml^{-1}, with nonspecific IgE forming up to a 10^4-fold excess. There are approximately 3×10^5 IgE receptors on normal rat mast cells[26]. Clearly, only a small proportion of these require to be occupied by EA-specific IgE to procure effective sensitisation, as in the parasitised animal most of the receptors will be occupied by IgE molecules with specificity for other antigens. Degranulation studies with the leukocytes of allergic humans show that only a few IgE cross-links, induced either by combination with antigen or anti-IgE antibodies, are required to cause histamine release[27,28], and evidently in our system the number of specific IgE molecules bound to the mast cells is such that they are in sufficient proximity to allow the requisite cross-links to be formed.

Thus, the ratio of nonspecific to specific IgE necessary to cause effective 'desensitisation' of mast cells is simply not achieved in helminth infection. Only perhaps in IgE myeloma will concentrations of nonspecific IgE be sufficient to ablate allergic reactions mediated by a normal IgE-antibody response. If allergies are found on balance to be less frequent in helminth-parasitised individuals, the reason is more likely to be found in other mechanisms.

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Antibody response to Moloney type C virus-induced tumours

Nancy Hogg & Diana Wallace

Tumour Immunology Unit, Department of Zoology,
University College, Gower Street, London WC1, UK

John E. Smart*

Imperial Cancer Research Fund, Lincoln's Inn Fields,
London WC2, UK

Animals infected with RNA tumour viruses show an immune response to new virus-induced antigens present on the tumour cell surface. In tumours induced by murine leukaemia virus (MuLV), serologically detected cell membrane antigens have been placed into two major groups; the Gross cell-surface antigen(s), GCSA and G_{IX}, are associated with Gross leukaemia virus infection[1] and the FMR antigen(s), associated with infection by Friend, Moloney or Rauscher type C viruses[2]. There is good evidence that some of these antigens are related to the structural proteins of the virus. The G_{IX} antigen is found on the viral envelope protein, gp70 (refs 3–5) and the GCSA antigen is on the p30 protein which is present in the membrane as part of the glycosylated 'gag' polyprotein(s)[6–8]. It is not certain whether the FMR antigen(s) is found on membrane-located viral structural precursors or polyproteins, or whether it is present on a tumour-associated protein. Several antigens of this latter type have been described. For example, the Moloney leukaemia virus-induced cell-surface antigen (MCSA) is thought to be a non-viral protein associated with 'gag' products, p30, p15 and p12 on the membranes of Moloney lymphoma cells[9,10]. MCSA and the antigens on cells infected by Abelson MuLV[11] may be very specific cellular antigens associated with Moloney MuLV infection. There has also been much interest in whether the *src* gene product is present in the membrane of sarcoma virus-induced tumours, although it is thought not to be present on the external membrane surface[12]. A more controversial point is whether there are other virus-induced cell-surface antigens (CSA) which may serve as targets for an effective immune response to viral tumours[13]. We have previously found[14] that serum from BALB/c mice with regressed Moloney virus-induced fibrosarcomas contained specificities only for Moloney viral proteins and not for any tumour-associated protein like MCSA. We have now identified the viral proteins with which the Moloney MuSV antisera react. Using a syngeneic system of immunisation with freshly induced Moloney sarcoma tumours, we show that the antibody response is directed only at viral structural antigens and that the major part of the response is to antigens on p30 protein probably present as part of a precursor polyprotein on the tumour cell surface.

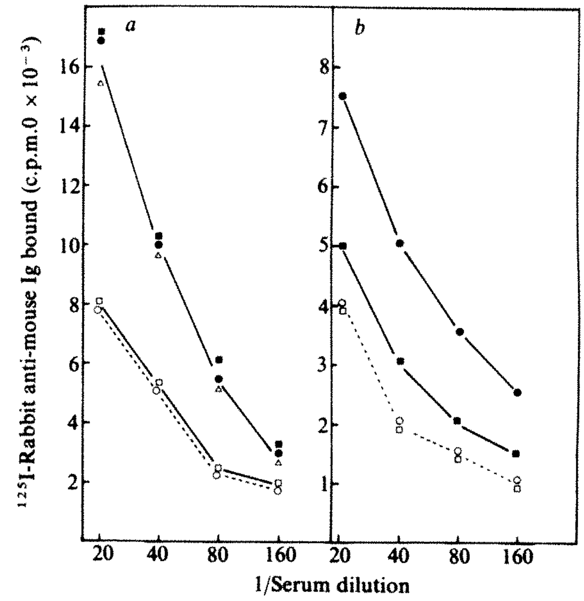

Fig. 1 Specificity of Moloney MuSV serum for type C virus-infected cells using cell monolayer radioimmunoassay. *a*, Moloney MuSV serum was reacted with secondary cultures of fibroblasts from Taylors Own (ICRF) mice, TO cells (□—□); MSC cells (●—●); TO cells infected 7 days previously with Moloney MuSV/MuLV virus contained in MSC supernatants (△—△); TO cells infected with sucrose banded Moloney MuSV/MuLV virus (■). Control BALB/c serum reacted with all cell preparations in a similar way (○---○). *b*, Comparison of binding of Moloney MuSV serum with MSC cells (●—●) and AKR-N3T3 cells (■—■) and of binding control BALB/c serum with MSC cells (○···○) and AKR.N3T3 cells (□···□). Samples were done in triplicate and standard deviations were always less than 10%.

The antisera used to describe several of the previously mentioned antigens have been obtained after an intensive immunisation schedule. By examining the response of BALB/c mice to regressing Moloney tumours which were induced by Moloney MuSV/MuLV virus rather than by tumour cells maintained in tissue culture, we hoped to obtain a closer approximation of immune responsiveness to a natural infection with virus. Thus, tumours raised in newborn mice by injection of purified Moloney MuSV/MuLV virus were subsequently injected intramuscularly into the flank of each of 20, 4-week-old BALB/c male mice. The resulting tumours regressed within 2 weeks. The serum used in this study, which will be called 'Moloney MuSV serum', represents pooled sera collected at the peak of the antibody response to tumour cells that occurred 18 days after tumour inoculation. A control group of sibling mice was maintained and bled coincidently with the tumour-bearing mice. The sera were adsorbed with BALB/c embryo fibroblasts before use.

In a cell monolayer radioimmunoassay[14], the Moloney MuSV serum binds to murine TO fibroblasts infected 1 week previously with Moloney virus from two different sources but does not bind

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* Present address: Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor, New York 11724.

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to any significant degree to uninfected fibroblasts (Fig. 1*a*). Control serum showed low levels of binding to both virus-infected and uninfected cells. Similarly, the Moloney MuSV serum bound to rat fibroblasts infected with Moloney virus, but not to uninfected rat fibroblasts (data not shown). As shown in Fig. 1*b*, the Moloney MuSV serum reacts with the MSC cell line[15], which is derived from a Moloney virus-induced sarcoma, and only to a small degree with Gross virus-infected N3T3 cells, called AKR-N3T3 cells[16]; this suggests that the Moloney MSV serum reacts chiefly with type-specific determinants induced by virus on Moloney virus-infected cells.

We have previously shown that the activity of this type of serum can be completely removed by adsorption of the serum with sucrose gradient Moloney MuSV/MuLV virus purified by sucrose gradient banding[14]. This has been confirmed in the present study and suggests that the major specificities in the Moloney MuSV serum are directed against antigens present on viral polypeptides expressed on the cell membrane. To assess which viral proteins were being recognised, disrupted ^{3}H-leucine-labelled Moloney MuSV/MuLV virus shed by the MSC cell line was precipitated with the Moloney MuSV serum. The Moloney MuSV serum precipitated predominantly p30 and gp70 proteins (Fig. 2*a*). Proteins coincident with p15(E) and p10/p12/p12(E) are precipitated to a minor degree and are therefore only faintly apparent in Fig. 2*a*. When the Moloney MuSV serum was adsorbed with Gross virus-infected AKR-N3T3 cells, the titre of antibodies reacting with p30 or gp70 was only slightly reduced (Fig. 2*b*), but when a similar adsorption was done with Moloney virus-infected MSC cells these specificities were completely removed (Fig. 2*c*). Control serum from unimmunised mice did not precipitate any viral proteins (data not shown). Therefore, we conclude that Moloney MuSV-induced fibrosarcomas induce antibody with specificities for Moloney type-specific determinants on p30 and gp70 proteins and that both are candidates for FMR antigens.

Adsorption of Moloney MuSV serum with Gross virus-infected AKR-N3T3 cells did remove some anti-p30 antibody and low titre antibodies reacting with the lower molecular weight viral proteins (Fig. 2*b*). These antibodies, as has been suggested by others[17–19], may be responsible for the cross-reactive or group specificity shared between the Moloney and Gross infected cells (Fig. 1*b*).

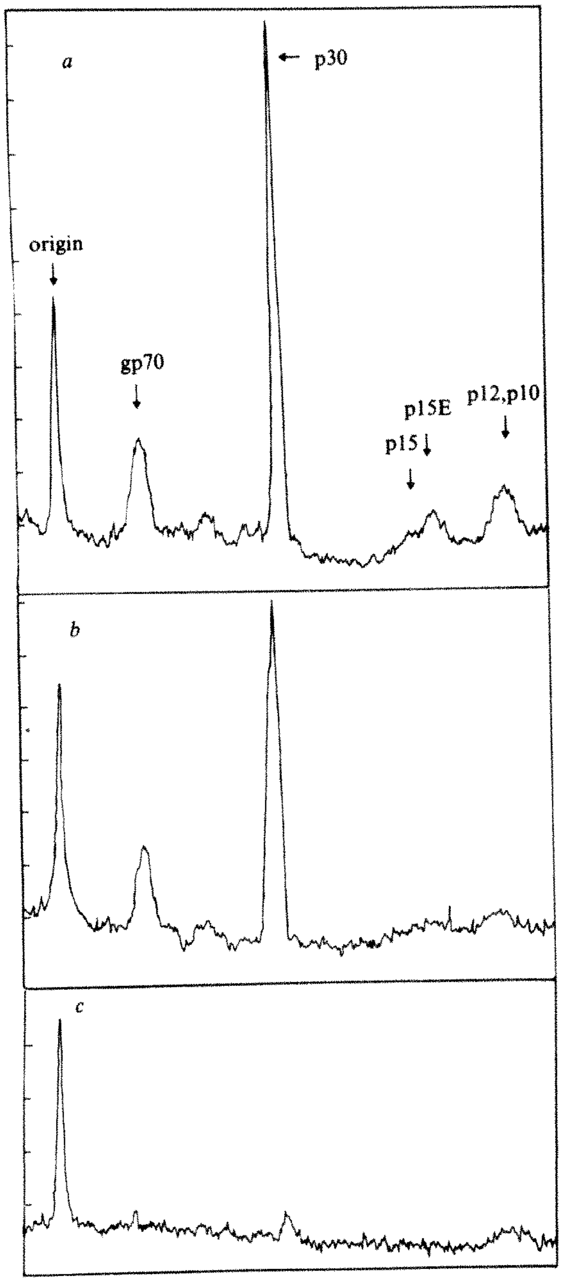

Fig. 2 Scanning densitometer tracings of an autoradiogram of 12% SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ^{3}H-leucine-labelled Moloney MuSV/MuLV. MSC cells were labelled overnight with radio-labelled virus purified from the supernatant by double banding in a sucrose density gradient and disrupted by freeze-thawing 10 times. Samples of virus containing 50,000 c.p.m. were reacted for 3 h with the appropriate antisera on ice. NP-40 was then added to a final concentration of 1% and the incubation was continued at 4 °C for 16 h. *Staphylococcus aureus* (formaldehyde fixed)[29] was then added and the incubation was continued for 1 h at 4 °C. The *Staphylococcus* A–IgG–antigen complexes were removed by low-speed centrifugation, the resulting pellets were then washed (resuspended and centrifuged) with 0.01 M Tris, *p*H 7.4, followed by 1% sodium deoxycholate, and finally 0.01 M Tris, *pH* 7.4. The samples were dissolved in SDS–sample buffer and applied to an SDS gel as previously described[30]. Adsorption of Moloney MuSV serum with cells was done by incubation of serum and cells at a ratio of 1:4 for 30 min on ice. Samples of ^{3}H-leucine-labelled Moloney MuSV/MuLV were precipitated by Moloney MuSV serum (*a*), antiserum adsorbed with AKR-N3T3 cells (*b*) and antiserum adsorbed with MSC cells (*c*). Standards used for calculating molecular weight were a sample of ^{3}H-leucine-labelled Moloney MuSV/MuLV and β-galactosidase (130,000), phosphorylase *a* (94,000), lactoperoxidase (80,000), catalase (60,000), alcohol dehydrogenase (41,000), carbonic anhydrase (29,000) and myoglobin (17,200) (not shown).

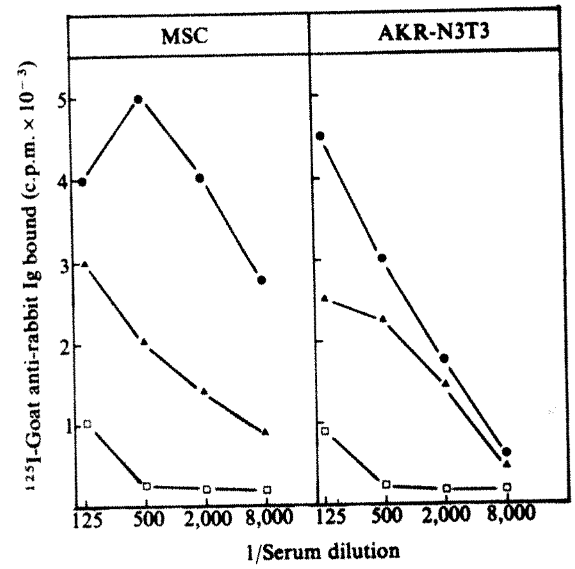

Fig. 3 Moloney MuSV/MuLV shedding MSC cells and the Gross MuLV shedding AKR-N3T3 cells were examined for expression of membrane type C viral antigens using a cell monolayer radioimmunoassay[14]. The cells were reacted with the following antisera to type C viral proteins[14]: rabbit anti-gp70 (●), rabbit anti-p30 (▲) and a control normal rabbit serum (□).

It was possible that AKR-N3T3 cells failed to adsorb p30 and gp70 antibodies from the Moloney MuSV serum because very little of these proteins were present on the membranes of the cells. To investigate this, rabbit anti-p30 and anti-gp70 reacting chiefly with shared or group-specific antigens[14] were titrated on both MSC cells and AKR-N3T3 cells. The titration profiles (Fig. 3*a*, *b*) show that both cell types have easily detectable amounts of both gp70 and p30 on their membranes. It is proposed that AKR-N3T3 cells fail to adsorb antiviral specificities from Moloney MuSV serum (Fig. 2*b*) because these antibodies are directed against Moloney virus type-specific determinants on both gp70 and p30 proteins.

FMR antisera have been shown to exhibit virus-neutralising activity and to contain antibodies which are cytotoxic for FMR-infected cells.[20] It has been reported that the cytotoxicity can be removed by adsorption of the antisera with an internal virion protein thought to be p15 (ref. 21). More recently, Nowinski and coworkers have provided evidence associating FMR antigenicity with the envelope protein, gp70 (ref. 22). In the present study, we have also found type-specific responses to gp70 protein but, in addition, we found higher titres of antibody directed against type-specific antigens on p30 protein. The finding of an immunogenic type-specific p30 antigen(s) as a possible component of the FMR antigen(s), suggests that this antigen is analogous to GCSA antigen induced by Gross virus infection, which is present on p30 as part of membrane bound 'gag' polyprotein. We do not know the molecular nature of the Moloney virus cell membrane p30. Many other species such as rats, cats, hamsters, rabbits and higher primates make antibody to p30 (refs 19, 23, 24) in response to type C virus infection. Mice have formerly been thought to be tolerant to p30 protein[25–27] unless immunised across strain barriers, as was done to raise the GSCA antisera[1]. Our study indicates for the first time that mice can mount a good response to p30 when immunised syngeneically and that the responses to G_{IX} and GCSA antigens on Gross virus tumours[1,28] and the FMR antigen on Friend, Moloney and Rauscher virus tumours are very similar in that both are type-specific responses to gp70 and p30, and not, in general, to other tumour-associated proteins.

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Anti-idiotypic antibodies in a patient with a functioning renal graft

Tetsuya Miyajima*, Reiko Higuchi*,
Hidechika Kashiwabara†,
Takeo Yokoyama† & Shigeyoshi Fujimoto‡

* National Cardiovascular Center, Research Institute, Suita, Osaka, Japan
† Sakura National Hospital, Ebara, Sakura, Chiba, Japan
‡ Laboratories for Immunology, School of Medicine, Chiba University, Chiba, Japan

A central problem in improving the success of renal transplantation in humans is to discover how to induce in the recipient an active immunological unresponsiveness to the donor's alloantigens[1–3]. There is increasing evidence that immunological unresponsiveness can be induced by either suppressor T cells[4,5] or antibodies directed against the T-cell receptor, that is, anti-idiotypic antibodies[6,7]. In our institutes, there are now six successful renal recipients. One of these, CD-S5, is a 32 year old man, HLA tissue-typed as Aw31, Bw39, Bw54 and DRw4. He received a second kidney transplant from a cadaveric donor having HLA-A2 and B5 on 21 August, 1975, after the first transplant from his brother with Aw26 and Bw40 had been rejected. The stimulation index of the mixed lymphocyte reaction (MLR) between patient and first donor was 11.9 but MLR could not be tested in the second grafting. One year after the second graft, immunosuppressive treatment with azathioprine was withdrawn because of drug toxic hepatitis. In spite of the administration of only prednisolone at 10 mg per day for the past 3 yr, the patient's creatinine clearance has remained at 70 ml min^{-1}. We proposed that the long survival of the renal graft might be due to an immunological unresponsiveness induced by the host immunoregulatory mechanism of this patient. To test this possibility, we have investigated the patient's serum for factor(s) capable of suppressing the host immune response. We report here our finding that the patient's serum contained an antibody against a T-cell receptor for a certain MLR, which was capable of specifically inhibiting a certain MLR.

First, we carried out an MLR inhibition test between recipient and donor using the recipient's serum. However, the recipient had received his graft from a cadaveric donor and the responsiveness of his lymphocytes in the MLR was found to be very weak, probably because they had already been inactivated, either by the host regulatory mechanism or by the immunosuppressive drug. A similar HLA-mismatched combination from a third individual was then attempted. As the HLA-B locus is more closely linked to the HLA-D locus (the MLR locus) than HLA-A, a combination of responder T.M. (A2, A11) (Bw15,) (DRw4,) and stimulator R.H. (A2, Aw26) (B5,) (DRw2,) cells was selected from healthy individuals who had the same mismatched HLA-B antigen B5 as was found between recipient and cadaveric donor. B5 antigen has recently been split into two antigens, Bw51 and Bw52, and B5 of the stimulator R.H. was later defined as Bw52. Using this combination, the patient's serum was subjected to the MLR inhibition test by its addition to the mixed lymphocyte culture. As shown in Table 1, the patient's serum clearly inhibited the MLR of this combination. The characteristics of the inhibitory factor in the patient's serum were then investigated to determine whether or not it was an antibody. For this, the patient's serum was fractionated by Sephadex G-200 column chromatography with borate-buffered saline, *p*H 8.0. Fractions of 19S, 7S, 4S and <4S, after being concentrated to the original volume by negative pressure dialysis, were each tested for their inhibitory activity in the MLR. Inhibitory activity was found mainly in the 7S fraction, and was

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Table 1 The effect of the renal recipient's serum fraction on the MLR of the selected third-party combination

Serum fraction added to the MLR	^{3}H-Thymidine incorporation by T.M. cells against R.H. (mean c.p.m. ± s.d.)
—	1,384 ± 252*
Not added	16,856 ± 1,234
Whole serum	1,480 ± 396
19S fraction	11,799 ± 1,099
7S fraction	3,371 ± 469
4S fraction	5,900 ± 899
<4S fraction	15,170 ± 769
7S fraction absorbed with rabbit anti-human IgG ab.	15,822 ± 1,177

The serum of the renal recipient was fractionated by elution on Sephadex G-200 column chromatography (2.6 × 100 cm) with borate-buffered saline, *p*H 8.0. As the inhibitory activity was mainly found in the 7S fraction, it was absorbed with purified rabbit antibodies directed against human IgG at room temperature for 1 h and then at 4 °C overnight at optimal ratio. Ten per cent of unabsorbed 7S fraction or absorbed 7S fraction was added to the culture medium of the selected MLR which had the same mismatched HLA-B antigen, B5, as found between recipient and cadaveric donor. Phenotype of responder T.M. is (A2, A11) (B15,) (DRw4,) and that or stimulator R.H. is (A2, Aw26) (Bw52,) (DRw2,). For MLR, 1×10^5 lymphocytes, prepared from defibrinated peripheral blood, were cultured with 1×10^5 2,000-rad irradiated stimulating cells for 5 days in microtissue culture plates (Nunclon N-1182, Roskilde) using RPMI-1640 medium (GIBCO) with 10% heat-inactivated human serum. Sixteen hours before collection, 0.5 μCi of ^{3}H-thymidine was added to each well and its incorporation measured with a liquid scintillation counter. Values are expressed as mean c.p.m. ± s.d. of triplicate samples.

* The value shows ^{3}H-thymidine incorporation by responding cells without stimulating cells.

completely removed by the absorption of IgG from the fraction using purified rabbit antibodies against human IgG at an optimal ratio (Table 1). This indicated that the inhibitory effect on the MLR is mediated by IgG antibodies. The serum IgG fraction was then isolated on DEAE-cellulose column chromatography by elution with 0.005 M phosphate buffer, *p*H 8.0, and tested for specificity of the MLR inhibition using various responder–stimulator cell combinations (Table 2). Treatment of responder cells (2×10^6 cells) with 0.5 ml of the antibody fraction (3 μg ml^{-1}) selectively inhibited the MLR of only those combinations with the same mismatched HLA-B antigen B5 as found in the combination of recipient and cadaveric donor. Also, the IgG fraction did not inhibit the MLR of the responder cell S.S., which differs from T.M., against any stimulating cells. Furthermore, the treatment of the stimulating cell R.H. with the antibody fraction had no effect on the response of untreated responder T.M. cell against the treated R.H. cell (Table 2), indicating that the inhibition is specific for very restricted combinations and is not mediated by antibody directed against common surface antigens of either the responder or the stimulator cell. In addition, the inhibitory effect on the MLR was directed against the responder cells, not against the stimulator cells.

The specificity of the inhibition by the antibody fraction was confirmed by an absorption study. When the antibody fraction (3 μg protein ml^{-1}) was absorbed three times with the responding cell T.M. (1×10^7) the inhibitory activity was markedly reduced, whereas absorption with an equal number of stimulator cell I.M. failed to remove the inhibitory activity (Table 3). Therefore, this restricted inhibitory effect on the MLR of specific pairs indicates that the antibody found in the patient's serum is directed against the T-cell receptor initiating MLR of the responding cell.

The nature of the T-cell receptor has been investigated in several laboratories[8,9]. Studies using anti-idiotypic antibody provide evidence that the T cell has receptors sharing idiotypes with the B cell for T-cell mediated immune reactions. Binz *et al.*[10] have already reported that the MLR, which is thought to be a T-cell mediated immune reaction[11], can be inhibited by an anti-idiotypic antibody directed against alloantibodies or T-cell receptors for alloantigens. An anti-idiotypic antibody has also been shown to compete with the corresponding alloantigen for binding to MLR-activated T-cell blasts[12], indicating that the inhibition of the MLR is mediated by the binding of anti-idiotypic antibodies to the antigen binding site of the T-cell receptor. The present finding that the inhibitory activity of the MLR in the recipient's serum is completely removed by absorption with purified rabbit antibodies directed against human IgG shows that the activity is associated with IgG antibody and directed against responder cells, but not stimulator cells. Moreover, the treatment of responder cell T.M. with the IgG antibody completely inhibited only the response against two types of stimulator cells, those which share Bw52 and DRw2,

Table 2 The effect of serum IgG antibody fraction from the renal transplant patient CD-S5 on various third-party MLR

		^{3}H-Thymidine incorporation (c.p.m. ± s.d.)		
Phenotype of responder*	Phenotype of stimulator*	Untreated	Treated responder	Treated stimulator
T.M. (2, 11) (15,) (4,)	—	1,288 ± 243	1,175 ± 181	—
	R.H. (2, 26) (52,) (2,)	16,392 ± 732	1,118 ± 220	20,220 ± 2,225
	I.M. (24,) (15, 52) (2,)	14,373 ± 2,883	1,969 ± 105	ND
	O.Y. (24,) (51, 54) (4,)	19,295 ± 1,181	22,679 ± 1,637	ND
	K.E. (11, 26) (12,) ( )	15,575 ± 1,657	19,756 ± 786	ND
	M.T. (2, 26) (40,) ( )	22,317 ± 773	21,988 ± 832	ND
	D.N. (2, 26) (40,) (4,)	17,016 ± 908	15,054 ± 1,696	ND
	M.Y. (2, 26) (15, 35) (4,)	12,475 ± 760	10,699 ± 2,831	ND
	M.N. (24, 31) (51, 52) (4,)	10,185 ± 1,042	12,903 ± 2,574	ND
	J.H. (11, 24) (13, 52) (2, 5)	15,394 ± 906	11,155 ± 936	ND
	T.H. (11, 24) (7, 15) (1, 4)	10,703 ± 174	12,387 ± 302	ND
	K.S. (2, 24) (35, 40) (2,)	19,110 ± 704	21,207 ± 3,155	ND
	S.T. (26, 31) (15, 52) (2,)	15,738 ± 346	11,044 ± 129	ND
	M.K. (2, 24) (15, 52) (2,)	16,858 ± 1,650	11,743 ± 1,520	ND
S.S. (33,) (12, 40) ( )	—	1,404 ± 324	—	—
	R.H. (2, 26) (52,)(2,)	11,674 ± 832	13,782 ± 603	ND
	I.M. (24,) (15, 52) (2,)	9,526 ± 307	8,458 ± 910	ND
	T.M. (2, 11)(15,) (4,)	13,307 ± 627	14,666 ± 674	ND

The IgG fraction was isolated from the patient's serum by DEAE-cellulose column chromatography using 0.005 M phosphate buffer at *p*H 8.0. 3×10^6 cells of either responder T.M. or stimulator R.H. were treated with 0.2 ml of the IgG antibody fraction (3 μg ml^{-1}) for 30 min at 37 °C and then washed three times with RPMI-1640 medium. After treatment, the responder cells were mixed with 12 different irradiated stimulator cells. In another experiment, R.H. stimulator cells were mixed with untreated responder cells T.M.

* The first parenthesis is for *A* locus, the second for *B* locus, and the third for *DR* locus antigens.

Table 3 Absorption of the inhibitory activity of the antibody fraction with the responder (T.M.) cells but not with the stimulator (I.M.) cells

		^{3}H-Thymidine incorporation by T.M. cells (mean c.p.m. ± s.d.) treated with			
Phenotype of responder*	Phenotype of stimulator*	None	Unabsorbed antibody fraction	Antibody fraction absorbed with T.M.	Antibody fraction absorbed with I.M.
T.M. (2, 11) (15,) (4,)	—	986±264	—	—	—
	R.H. (2, 26) (52,) (2,)	8,836±266	423±178	8,352±972	1,967±575
	I.M. (24,) (15, 52) (2,)	7,443±735	773±115	7,553±768	1,758±291
	S.S. (33,) (12, 40) (,)	9,603±428	7,715±215	8,911±700	8,461±244

The IgG antibody fraction had an inhibitory effect on the MLR even at a concentration of 3 μg ml^{-1}. Therefore, 0.5 ml of 3 μg ml^{-1} of the IgG antibody fraction was absorbed with either 1×10^7 I.M. stimulator cells or 1×10^7 T.M. responder cells for 30 min at 37 °C. The absorption was repeated three times with the same number of fresh cells each time. After absorption, the IgG fraction was tested for its inhibitory effect on the MLR, treating the responder cells T.M. with either unabsorbed or absorbed IgG antibody fraction.

* The first parenthesis is for *A* locus, the second for *B* locus and the third for *DR* locus antigens.

but did not inhibit the response against 11 other types of stimulating cells. This suggests that the antibody is directed against a restricted clone of T cells bearing the MLR antigen of the stimulating cells R.H. and I.M. From this, and observations reported by others, we consider that the patient's serum contains antibody against a T-cell receptor for an MLR antigen, that is, an anti-idiotypic antibody. Genotype for the responder cell T.M. is *A2, /A11, Bw15, DRw4*; for the stimulating cell R.H., *A2, /Aw26, Bw52, DRw2*; and for the stimulator cell I.M., *Aw24, Bw15, /Aw24, Bw52, DRw2*. Phenotype of the cadaveric donor of the patient was (A2,) (B5,). From these combinations, because one haplotype of stimulator R.H. or I.M. is in parts identical to that of the responder T.M., we suggest that this antibody could bind an idiotype of the receptor specific for DRw2-associated MLR determinants.

In contrast to our findings, it has been reported that in experimental animals, idiotypes of T cells usually differ between strains, although intrastrain idiotypic cross-reactions of T-cell receptors are extremely strong[13]. However, it has been observed that idiotypes are shared in immunoglobulins from genetically different individuals[14]. Furthermore, T-cell receptors are generally idiotypically more homogeneous than antibodies[15,16]. These findings suggest that certain idiotypes are shared by a T-cell clone of T.M. and the renal recipient, which can be activated with DRw2-associated MLR antigen.

As presented by Jerne, the immune network theory claims that the autoimmune response to self-idiotypes can basically act as an immunoregulatory system[17]. The passive immunisation with an anti-idiotypic antibody or active immunisation with parental cells of F_1 hybrids induces immunological unresponsiveness in animals against the relevant antigen[6,18]. This idea seems attractive for clinical transplantation, and it is conceivable that anti-idiotypic antibodies directed against autologous idiotypes on T cells are at least partly responsible for the successful graft survival in the patient not receiving azathioprine.

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Stimulation of cytolytic T cells by isolated viral peptides and HN protein coupled to agarose beads

Daniel P. Guertin & David P. Fan

Department of Genetics and Cell Biology, University of Minnesota,
1445 Gortner Avenue, St Paul, Minnesota 55108

Sendai virus-infected mouse cells can be lysed by mouse cytolytic thymus-dependent lymphocytes (CTL) directed specifically against the infected cells. The CTL is known to recognise the H–2 antigens on the target cells together with structure(s) including at least the two viral surface glycoproteins also found on purified virus[1–8]. We report here that anti-Sendai CTL can be stimulated *in vitro* by detergent-solubilised viral haemagglutinin–neuraminidase (HN)[9], either as the isolated protein or coupled to agarose beads. We further show stimulation by the hydrophilic portion of a protein removed from the virus by the protease subtilisin BPN′, and we demonstrate that cyanogen bromide- (CNBr-) cleaved viral peptides also produce such stimulation.

For studies with the detergent-solubilised Sendai glycoproteins we made three preparations: (1) a mixture of HN and the fusion protein (F)[9,10], denoted HNF, contaminated by a small amount of nucleoprotein (NP)[9], or a breakdown product of HN[19]. (2) cleanly purified F, and (3) HN fraction separated completely from the F but retaining a trace amount of NP or HN breakdown material (Fig. 1). Since the NP is normally associated with the nucleic acid at the interior of the virus[9], we assume that this protein is not likely to be a CTL antigen. Thus any small amount of NP in the HN and HNF preparations should not affect their properties in CTL stimulation.

The preparations of HNF, F and HN were either: (1) left untreated, (2) dialysed to remove detergent, or (3) coupled covalently to *N*-hydroxysuccinimide-activated agarose beads at initial concentrations ranging from 25 to 60 μg protein per 0.5–6.5 ml packed beads in a total volume of 3–15 ml. In preliminary experiments, the HNF and HN, either free or bound to beads, were consistently able to stimulate H–2-restricted anti-Sendai CTL *in vitro* from spleen cells of BALB/c(H-2^{d}) and C57BL/6(H – 2^{b}) mice previously exposed to Sendai virus. The BALB/c CTL were typically able to lyse P815(H-2^{d}) mastocytoma cells only after Sendai infection and were unable to lyse EL4(H – 2^{b}) leukaemia cells either with or without Sendai infection. In contrast, HNF- and HN-stimulated C57BL/6(H-2^{b}) CTL specifically lysed infected EL4(H-2^{b}) but not P815(H-2^{d}) cells either with or without infection. There did

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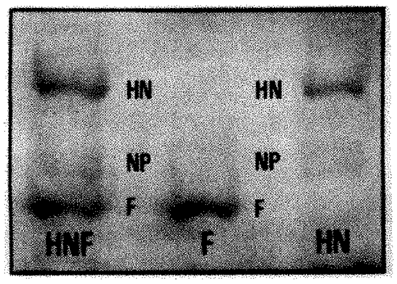

Fig. 1 Preparation of Sendai F, HN and HNF and their presentation to mouse spleen cells for CTL stimulation *in vitro*. SDS-polyacrylamide gel electrophoresis of Sendai protein fractions. Purified Sendai virus was extracted with 0.25% Triton X-100 in 0.01 M sodium phosphate buffer, *pH* 7.2, at 6.4 mg protein ml^{-1} for 30 min at room temperature with repeated mixing in a homogeniser. After centrifugation at 200,000*g* for 1 h (ref. 9), the HNF was contained in the supernatant of the extracted proteins. The yield of HN was 20% as measured by neuraminidase enzyme assay. The HN fraction was HNF material which did not absorb to a diethylaminoethyl-agarose (DEAE-BioGel A, BioRad) column equilibrated with 0.1% Triton X-100 in 0.01 M sodium phosphate buffer, *pH* 7.2 (ref. 16). The F fraction was HNF material which did not absorb to a hydroxyapatite (BioGel HTP, BioRad) column equilibrated with the same buffer. The yield of both F and HN was over 80% from the two columns. The purified HN had 460 units neuraminidase per μg protein. Aliquots of 15 μg HNF, 6 μg F and 9 μg HN were loaded onto the 7% gel[17].

tend to be some stimulation of C57BL/6 CTL against uninfected EL4 cells although this stimulation was always less marked than that for the infected cells. These characterisation experiments showed that the fact that the BALB/c CTL did not lyse the Sendai infected EL4 cells was not due to the inability of the infected EL4 cells to act as targets for anti-Sendai CTL. On the contrary, the CTL stimulated by the isolated viral proteins gave the typical H-2 restriction seen for anti-Sendai CTL[1-8]. In view of the more clearcut viral dependence with the BALB/c CTL, further experiments were performed with this system.

Results with the isolated F protein, either free or on agarose beads, were inconsistent, at best showing marginal stimulation; so experiments with the F protein were not pursued further. However, F is in fact an antigen recognised by anti-Sendai CTL since the F and H-2 proteins inserted together into lipsomes can stimulate these CTL although the antigenicity seems to be lower than that for HN[8].

The extent of stimulation by the HN and HNF preparations was closely correlated to the amount of neuraminidase and hence HN in the preparations regardless of whether the antigen was free in a detergent-containing solution, had been removed from the detergent by dialysis, or had been coupled to agarose beads (Fig. 2). The same maximum stimulation was observed with 0.04–0.80 units neuraminidase per well and 130–16,000 beads per well. The minimum amount of HN needed to give maximum stimulation represented enzyme from 30 virus particles per cell. The presence of the extra F in the HNF preparations did not increase stimulation beyond that of HN alone, confirming that the F had little if any stimulatory activity. The

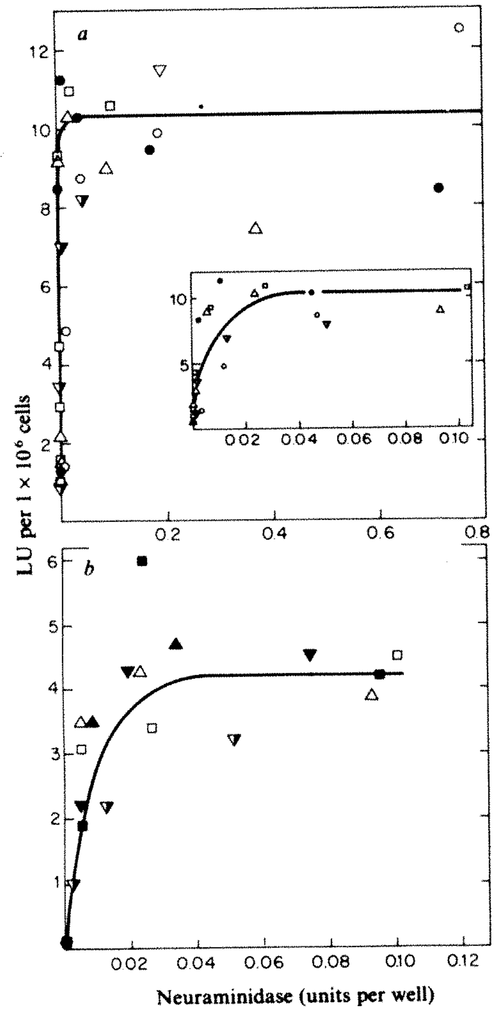

Fig. 2 Dependence of anti-Sendai CTL stimulation on HN concentration. The HNF and HN preparations of Fig. 1 were shaken gently overnight in the cold with *N*-hydroxysuccinimide-activated agarose beads (2×10^6 packed beads ml^{-1}, bead diameter 50–200 μm, Affigel-10, BioRad) in 0.1 M HEPES buffer, *pH* 6.5. The covalently coupled beads were then washed with 0.1% Triton X-100 in 0.01 M sodium phosphate buffer to remove unattached protein, and unreacted *N*-hydroxysuccinimide groups were blocked by further shaking in 0.1 M glycine ethyl ester, *pH* 8.0 in 0.1% Triton X-100 for 1 h. The beads were finally washed repeatedly and suspended in TD buffer (8g NaCl, 0.38g KCl, 0.1 g Na_2HPO_4, 3.0 g Tris-HCl per litre, *pH* 7.4). (In the descriptions below involving the coupled beads, the concentrations at the begining of the coupling are indicated in brackets in μg ml^{-1} for protein and beads per ml $\times 10^5$ for the Affigel.) From neuraminidase enzyme measurements, the binding of HN to beads for HN(12)–Affigel(3) was 12% to give 4.5 units neuraminidase per 10^5 beads. The corresponding values for HN(5)–Affigel(6) were 29% and 2.8 units, for HN(2)–Affigel(7) were 43% and 1.3 units, for HNF(20)–Affigel(3) were 14% and 6.6 units, for HNF(10)–Affigel(6) were 20% and 2.4 units, and for HNF(4)–Affigel(7) were 51% and 1.9 units. For the stimulation of anti-Sendai CTL, spleen cells ($3–4 \times 10^6$) from BALB/c (H-2^d) mice immunised intraperitoneally several weeks previously with live Sendai virus were added to 16-mm diameter tissue culture wells (Linbro Scientific) together with the additions indicated in 0.5 ml serum-free medium. After 30 min at 37 °C, serum containing medium was added to give 2.5 ml per well at the final composition used previously[18]. The CTL in the wells were assayed as described[18] for the ability to release radioactivity from ^{51}Cr-labelled P815 (H-2^d) cells exposed to an excess of UV-inactivated Sendai virus in a small volume of serum-free medium for 1 h. The lytic units per 10^6 cultured cells (LU per 10^6) were as defined previously[18], except that lysis was measured after 6 h instead of 3 h. *a*, The inset has the same data plotted on an expanded scale. The antigens added to the wells were: the HN preparation as in Fig. 1 in detergent solution (○), the same preparation dialysed extensively against TD buffer to remove the Triton X-100 (●), HN(12)–Affigel(3) (△), HN(5)–Affigel(6) (□), and HN(2)–Affigel(7) (▼). Two control cultures stimulated with an excess of UV-inactivated Sendai virus gave 94 and 176 LU per 10^6 respectively. The inset shows the same data plotted on an expanded scale. The antigen added to these wells were the same HN–Affigel preparations used above (same symbols), plus: HNF(20)–Affigel(3) (▲), HNF(10)–Affigel(6) (■), and HNF(4)–Affigel(7) (▼). Two control cultures similar to the ones used in *a* gave 70 and 119 LU per 10^6 respectively.

anti-Sendai CTL were indeed thymus-dependent because lysis could be reduced more than 99% by treatment with anti-Thy 1.2 antibody and rabbit complement.

Since the haemagglutinin activity of the HN will cause the protein to bind to cell surfaces, it was conceivable that the protein was acting as a lectin in the same way as concanavalin A (Con A), which is known to stimulate CTL nonspecifically[11]. This possibility was unlikely since HNF beads did not efficiently stimulate anti-H-2^d specific CTL from C57BL/6(H-2^b) mice previously immunised with H-2^d cells, while Con A on beads was able to do so (Fig. 3). Conversely, Con A on beads did stimulate these allospecific CTL but not the anti-Sendai CTL as the HNF beads had done. The ability of bead-bound Con A to stimulate at least one class of T cells is in contrast to a previous report[12] that Con A bound to surfaces could stimulate B but not T cells. It is conceivable that only a small subset of T cells could be stimulated by Con A on beads and that this subset was missed by the earlier report.

The results with the bead-bound antigen suggested that stimulatory antigen need not be presented to spleen cells in the context of a lipid membrane. The ability of antigen coupled to beads to stimulate CTL has also been demonstrated for H-2 alloantigens[13]. Next, we tested for stimulation by fragments of Sendai proteins, beginning with those cleaved from the virus by subtilisin BPN′. In the conditions used, the HN was liberated from the virus as demonstrated by mobility of the neuraminidase activity at a rate corresponding to a molecular weight smaller than bovine serum albumin (molecular weight 68,000) on an agarose gel filtration column (Fig. 4*a*).

Since our object was to determine the minimum antigenic determinant for anti-Sendai CTL stimulation, we chromatographed the subtilisin-solubilised viral proteins on a Sephadex G-75 column with an exclusion limit of molecular weight 70,000 and tested the fractions for both neuraminidase activity and CTL stimulation (Fig. 4*b*). The enzyme activity appeared almost in the excluded volume, indicating that the solubilised HN had a larger apparent molecular weight on this column than on the agarose column. Anti-Sendai CTL stimulatory material was found in the region where the HN protein and smaller materials ran.

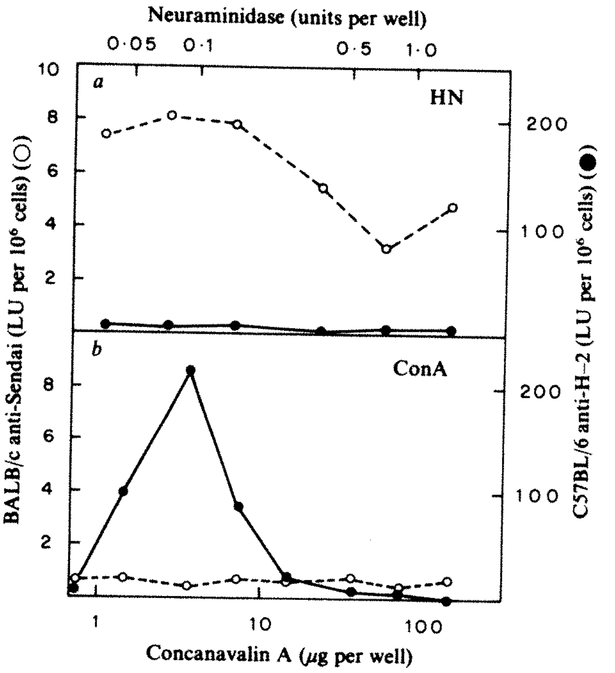

Fig. 3 Specificity of HNF stimulation of CTL. *a*, BALB/c(H-2^d) spleen cells were stimulated with HNF(4)–Affigel(14) at various concentrations and assayed for anti-Sendai CTL using Sendai-infected P815(H-2^d) target cells as described in Fig. 2. Spleen cells from C57BL/6(H-2^b) mice immunised intraperitoneally several weeks previously with 30×10^6 P815(H-2^d) cells were also stimulated *in vitro* as above with HNF(4)–Affigel(14) and were assayed for anti-H-2^d CTL using the same Sendai-infected P815 target cells. The assay was for 3 h instead of 6 h. The results were essentially the same when non-infected P815 target cells were used. *b*, The same conditions were used in the stimulation and assay except that concanavalin A covalently coupled to agarose beads at 15 mg ml^{-1} beads (Miles Labs) was used in place of the HNF(4)–Affigel(14).

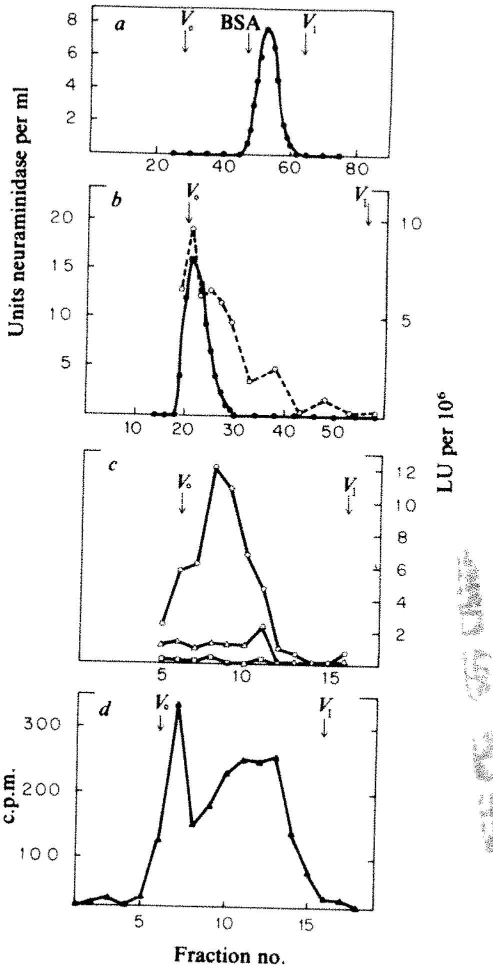

Fig. 4 Characterisation of subtilisin and CNBr-cleaved fragments of Sendai viral proteins. Purified Sendai virus was digested for 60 min at 37 °C with 100 μg subtilisin BPN′ (Sigma) (100 μg per 30 mg viral protein per ml) in 0.005 M sodium phosphate buffer, *p*H 7.4. After digestion, the sample was centrifuged at 200,000*g* for 60 min, and the supernatant solution containing approximately 10% of the neuraminidase activity in the original virus preparation was chromatographed on a Biogel A-5 m (BioRad, 11 mm × 110 cm) gel filtration column packed with 6 mm diameter glass beads (*a*) and on a Sephadex G-75 (Pharmacia, 21 mm × 55 cm) gel filtration column (*b*) using 0.005 M sodium phosphate buffer, *p*H 7.2. The subtilisin-solubilised material was also mixed with one-half volume of formic acid and CNBr equivalent to 100 times the estimated mass of the solubilised protein. After overnight cleavage at room temperature, the sample was lyophilised, resuspended in 0.2 M acetic acid chromatographed on a Sephadex G-25 (Pharmacia, 6 mm × 25 cm) gel filtration column in 0.2 M acetic acid (*c*). A separate virus sample (*d*) was subtilisin- and CNBr-digested and then chromatographed on a G-25 column as in *c* except that the starting virus was labelled radioactively using ^{3}H-D,L-alanine injected into the allantoic cavity of the egg used for viral growth. The void (V_0) and included (V_I) volumes of the column are indicated for each chromatogram. The samples from the columns were analysed for neuraminidase activity (●), radioactivity (▲), and the ability to stimulate CTL. The column fractions were lyophilised, resuspended in TD buffer, and added directly to the cultures for the *in vitro* stimulation as described for Fig. 2. The assays were also as in Fig. 2 except that the target cells included P815(H-2^d) non-infected cells (△), and EL4(H-2^b) Sendai-infected cells (□) in addition to the P815 Sendai-infected cells (○).

Thus it was conceivable that fragments of the HN too small to have neuraminidase activity could still stimulate CTL. After subtilisin-solubilised viral proteins were further cleaved with CNBr, over 99% of the neuraminidase activity was abolished. The cleavage products from radioactively labelled virus were found to have molecular weights ranging from 1,000 (the inclusion limit of Sephadex G-25) to over 5,000 (the exclusion limit of the same gel filtration beads) (Fig. 4*d*). Fractions from the G-25 column corresponding to proteins smaller than the excluded volume were still able to stimulate BALB/c anti-Sendai CTL (Fig. 4*c*). Furthermore, these CTL were Sendai-specific and H-2-restricted, lysing H-2^{d} but not H-2^{b} cells only after Sendai infection (Fig. 4*c*).

The material from fractions 19–30 from the column of Fig. 4*b* was also pooled, cleaved with CNBr and chromatographed on the G-25 column used in Fig. 4*c*. The same pattern of stimulatory material was observed indicating that compounds of small molecular weight could indeed be generated by CNBr cleavage of larger molecules and retain the stimulatory capacity. When the starting material for the CNBr cleavage from the pooled fractions was analysed by sodium dodecyl sulphate–polyacrylamide gel electrophoresis as described in Fig. 1*a*, only two bands were seen at molecular weights of approximately 55,000 and 61,000. Because of their large sizes, neither could have been derived from the F glycoprotein. Furthermore, protein corresponding to the smallest detergent-solubilised F peptide (F$_1$)[10] at molecular weight 51,000 or less was not seen suggesting that no material from the F protein was represented in these G-75 column fractions. However, at least one of the new bands was likely to have been derived from the larger HN by proteolytic cleavage, since neuraminidase activity was still present and the band corresponding to detergent-solubilised HN at molecular weight 69,000 was missing.

In a comparison experiment, the maximum stimulations by the subtilisin-solubilised viral peptides for Sendai-infected P815 target cells, non-infected P815 targets, and Sendai-infected EL4 targets from five independent samples in the plateau region of saturating antigen were 8.4 ± 1.9, 2.4 ± 0.5 and 0.7 ± 0.2 LU per 10^{6} cells, respectively. The corresponding values for the CNBr cleaved material were 5.6 ± 1.7, 1.6 ± 0.3 and 0.6 ± 0.2 LU per 10^{6} cells, respectively, again for five samples in the plateau region. Antigen from fraction 9 of Fig. 4*c* of molecular weight less than 5,000 was also able to stimulate with corresponding values of 7.3 ± 0.8, 1.4 ± 0.2 and 0.4 ± 0.1 LU per 10^{6} cells, respectively. In all cases, the maximum stimulation was similar to that for the detergent-solubilised HN that is, in the range of 5–10% of the stimulation found for UV-inactivated Sendai virus. Within the limits of sample loss, the CTL stimulation was as efficient using CNBr fragments as it was with the subtilisin-cleaved HN. Therefore, it does not seem that any of the antigenic determinants for this low level of stimulation were destroyed by the subtilisin and CNBr treatments. It is possible that this low level of stimulation corresponds to that found by Finberg, Mescher and Burakoff[2] using liposomes containing viral proteins in the absence of any H-2 products and probably derives from a special subset of all anti-Sendai CTL precursors. The ability to use isolated viral proteins to stimulate anti-viral CTL has also been reported for influenza virus[14,15].

The advantage of using bead-bound antigens stems from the fact that the beads are large and can be removed easily and quantitatively from the *in vitro* stimulation mixture so that the time course of the stimulation can be studied precisely. In initial experiments, the beads lost all their ability to stimulate fresh cells after overnight incubation with responder cells. Therefore, it is conceivable that the antigen bound to the beads was cleaved from them during the incubation and that antigen fragments constituted the actual stimulatory material. Alternatively, the bead-associated antigen could have been processed for the stimulation in some other way. Also, a variety of cells, including macrophages, can be exposed to the beads to determine which cells process the added antigen first. The use of protein fragments in the stimulation tests should permit the biochemical characterisation of the minimum antigenic determinants for CTL stimulation. As a working hypothesis, we are assuming that one or more cleavage products of HN are responsible for the stimulation since stimulation by the purified detergent-solubilised proteins correlated most closely with the presence of this protein, and, also, analysis of the G-75 column made it unlikely that the F protein was present in fractions which gave rise to stimulatory fragments.

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Viral pathogenesis and resistance to defective interfering particles

Steven Jacobson & Charles J. Pfau

Department of Biology, Rensselaer Polytechnic Institute, Troy, New York 12181

Recently presented evidence indicates that synthesis of defective interfering (DI) particles following infection of cultured cells with lymphocytic choriomeningitis (LCM) virus may result in self-curing[1]. The genesis of DI particles can occur in cells infected with virtually any animal virus[2], and seems to be a host-controlled function[3]. Thus, these particles may represent a third line of defence—with the immune response and interferon—against virus invasion[3,4]. LCM virus infection causes the rapid appearance of DI particles in tissue culture cells[5,6]. Nevertheless, persistent infections can be easily established with LCM and other arenaviruses[7–9]. The LCM-persistent infections are apparently maintained in these cultures by the genesis of DI-resistant viruses[1]. This latter finding prompted us to determine whether such DI-resistant viruses occur in nature. Vertical transmission of LCM virus in the house mouse or neonatal infection of laboratory mice takes the form of a life-long inapparent infection. These mice lack functional virus-specific T lymphocytes, but may make an antibody response against the invasion. It is known that LCM virus injected into a neonatal mouse can change during the course of the infection so that at least two different viral populations coexist in the same mouse[10]. DI particles are produced in abundance shortly after neonatal infection and this precedes the appearance of a virus population that, by plaque morphology, differs from the one originally inoculated[11]. We report here the isolation of an LCM virus from persistently infected mice that is resistant not only to the DI particles which were produced shortly after neonatal infection with heterotypic virus but also to the DI particles that this virus itself induces in neonates. We know of no other report of viruses which can replicate normally in the presence of the DI particles they induce.

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A recently isolated clone of the UBC strain of LCM, designated substrain T, was injected into outbred (ICR) mice less than 24 h old. This virus strain had a long history of tissue culture passage in our laboratory[12]. Ten months later, the blood of these mice exclusively contained two virus types which were easily distinguishable from substrain T (Table 1). These cloned viruses were called substrains A and D because the disease syndromes they produced in mice infected as adults were similar (Table 1) to two previously described substrains of LCM-UBC designated 'aggressive' and 'docile' (ref. 13). All three virus types: (1) were neutralised by heat-inactivated (56 °C for 30 min) filtered ascites fluid taken from a patient infected with the MP strain of LCM; (2) induced patching and capping on living MDCK cell membranes after a fluorescein isothiocyanate indirect staining sequence using sera from a substrain T hyperimmunised hamster; (3) initiated life-long (>9 months) persistent infections when injected into neonatal ICR mice. DI particles for each of the three substrains, designated DI_T, DI_A and DI_D, were generated in neonatal mice by previously described methods[11]. Each newborn mouse in a litter (usually 15) was injected intraperitoneally with 100 LD_{50} of one of the substrains. Eight days later the mice were killed and kidneys from the littermates were pooled and homogenised. After resuspension in 10 ml of tissue culture medium and clarification by low-speed centrifugation, the putative DI particle stocks were stored at −70 °C until use. DI particle activity was measured in MDCK cells. These cells are killed with single hit kinetics by infection with standard virus. However, co-infection with a single DI particle not only spares the cell but also inhibits the replication of standard virus[14]. Our test was to infect a series of MDCK monolayers with one type of DI particle and then challenge them individually with each of the three virus substrains shown in Table 1 (which by themselves would cause almost complete destruction of the monolayer). DI particle activity was determined visually and by reduction of the amount of infectious challenge virus synthesised.

In each case, all three putative DI particle preparations completely inhibited any observable cytopathic effect (CPE) caused by standard virus substrains T and D (Table 2). Synthesis of these challenge standard viruses in the above conditions varied from 13% to 43% of normal (we have previously shown[14] that suppression of CPE and inhibition of standard virus synthesis by DI_T particles are separable functions). However, none of the DI preparations protected cultures challenged with substrain A (Table 2). Thus, substrain A seems to be relatively resistant to DI particles in general, even to those that it induced.

The results presented in Table 2 were judged to be due to DI particles for the following reasons. (1) Kidneys taken from normal mice had no sparing ability on cultures challenged with any of the three standard viruses. (2) UV treatment of the DI particle-containing kidney extracts in conditions which lowered the infectivity of standard virus 100-fold (multiplicity of infection (MOI) reduced from 0.7 to 0.007) had little or no effect on the cell-sparing phenomenon or the inability of these cells to support the replication of challenge virus (Table 2). This agrees with the previous observations of the relative UV resistance of LCM DI particles[15,16]. Furthermore, UV treatment did not increase the interference phenomenon, thus eliminating the possibility that it was an artefact created by irradiation of standard virus. (3) LCM-specific antiserum treatment of the kidney extracts taken from mice infected with substrain T lowered the standard virus titres (and supposedly the DI particle titres, as its polypeptide composition is not recognisably different from that of standard virus[17]) by 90% and effectively eliminated the cell-sparing and virus inhibitory properties. These results, combined with the unique properties of the DI_A particles, eliminated interferon as an explanation of the results. (4) Organ extract addition immediately after a 1.5-h adsorption period with 'challenge' virus still exerted its inhibitory effect on the virus, albeit somewhat less effectively[14]. This eliminated the possibility that the observed effects were due to blocking of receptor sites for the challenge virus or neutralising antibody in the kidney extracts. All these results are consistent with the general properties of DI particles[2]. When it becomes possible to separate LCM DI particles from standard virus effectively by physical means, purification of the DI particles from the kidney extracts can be undertaken.

Table 1 Plaque morphology and mortality pattern as a function of LCM virus substrain

Virus designation	MDCK plaque type* (diameter)	Murine characteristics† Appearance at death	% Dead	Mean day of death ± s.d. (range)
T	Clear (3–4 mm)	Convulsive	100	9.7 ± 1.4 (7–11)
A	Pinpoint (1-2 mm)	Convulsive	100	6.75 ± 0.5 (6–7)
D	Clear (3–4 mm)	Wasting	75	12.3 ± 5.8 (9–19)

* Monolayers were stained with neutral red on the fourth day after infection and plaques were scored within 24 h.

† Groups of 12 ICR mice, 14–21 days old, were injected intracerebrally with 100 LD_{50} of virus stock in a volume of 0.02 ml. Although a clear LD_{50} end point was obtained with substrain D, it was routinely observed that mortality fell as the dose of the inoculum increased.

Table 2 Ability of DI particles to interfere with the synthesis of different standard LCM viruses

Standard challenge virus subtype	% Of control challenge virus yield after pretreatment of MDCK cells with: DI_T*	DI_T	DI_A	DI_D
A†	95	90	94	94
D‡	13	32	23	26
T‡	25	30	43	37

Kidney extracts, which were found to contain the highest amount of DI particles after neonatal infection[11], were diluted so that 3 ml of tissue culture fluid contained the equivalent amount of virus extractable from one organ. Aliquots of 1 ml of the virus were then used to infect a 25-cm^2 monolayer of MDCK cells as described previously[14], or first subjected to the UV irradiation from two 30-W GE UV lamps giving an incident energy dose at the surface of the fluid of 6×10^4 erg. After a 1.5-h adsorption period the cells were repeatedly washed and then challenged with standard virus at an MOI of 0.1. The cultures were observed under the microscope at intervals over a 72-h period. DI particle-treated cultures were always compared with those that had either no pretreatment or pretreatment with organ extracts from normal mice. All tissue culture fluids were changed at 40 h post-infection and saved for future plaque assay titration. Control challenge virus yields were: substrain A, 1.3×10^8 plaque forming units (PFU) per ml; substrain D, 4.6×10^8 PFU per ml; substrain T, 3.0×10^8 PFU per ml.

* This preparation was not UV irradiated.

† All DI particle-pretreated cultures challenged with substrain A developed cytopathology to the same extent as cultures infected with substrain A alone.

‡ All DI particle-pretreated cultures challenged with substrains D or T developed little, if any, cytopathology.

Tissue culture models[1,5] of LCM persistent infections continue to have predictive value when applied to *in vivo* situations. The present finding of a DI-resistant virus offers an explanation for one of the plaque morphology changes in the virus as life-long infection progresses. The virus–cell interactions in these mice are obviously complex. The role of LCM substrain D is unclear; the time course of its appearance with regard to substrain A is under investigation. An LCM virus which changes slightly, if at all, in plaque morphology has also been observed in persistently infected mice[18]. This virus may initially have been resistant to DI particles, or evolved to this state without other striking signs of change. Our studies suggest that it may be worthwhile to determine whether DI-resistant viruses have arisen through natural selection in other persistent infections, especially those in which mutant viruses have appeared.

It has been proposed that cells budding LCM virus in the choroid plexus, ependyma and meninges are specifically recognised and damaged by sensitised T cells[19,20]. As DI particles suppress surface antigen expression in LCM-infected cells[21,22], the pathogenetic mechanisms of various LCM viruses in the adult mouse may be linked to their resistance to DI particles.

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^{1}H NMR study of valinomycin conformation in a phospholipid bilayer

G. W. Feigenson & P. R. Meers

Section of Biochemistry, Molecular and Cell Biology,
Clark Hall, Cornell University, Ithaca, New York 14853

Our understanding of how ions pass across biological membranes has been greatly advanced by the study of small molecules which are capable of enhancing ion transport. The concepts of ion movement through channels or via mobile ion carriers have arisen from studies of model systems[1–4]. However, direct probing at the molecular level of the process of ion movement in a membrane system has proved difficult. The electrical properties of black lipid membrane model systems do not provide information about the details of ionophore location or conformation. Spectroscopic methods which are suited for probing the details of ionophore conformation and the stoichometry of ion binding have been confined largely to organic solvent systems which are limited as models for biological membranes. We report here proton magnetic resonance (^{1}H NMR) spectroscopic studies which investigate valinomycin conformation and ion binding in small bilayer vesicles.

Valinomycin is a cyclic 12-membered depsipeptide composed of D-valine, L-lactic acid, L-valine and D-hydroxyisovaleric acid with the structure [D-Val-L-Lac-L-Val-D-HyIv]$_3$. It is an interesting ionophore because of its ion binding selectivity. It is particularly suitable for the work reported here because its conformational states are now well understood, largely owing to extensive NMR[5–8], circular dichroism[6,9], and infrared[6,9] studies in solvents, X-ray diffraction studies of crystals[10,11], and conformational energy calculations[12,13]. The present studies in phospholipid bilayers were made possible by our recent synthesis of a perdeuterated phospholipid, dimyristoyl phosphatidylcholine (DMPC-d$_{72}$) (ref. 14). DMPC-d$_{72}$ has 99.0–99.7% replacement of ^{1}H by ^{2}H throughout the molecule. The corresponding reduction in the ^{1}H NMR background signal makes it possible to observe ^{1}H NMR spectra from small molecules which are incorporated into bilayer vesicles of the perdeuterated lipid[15].

DMPC-d$_{72}$ was synthesised according to Kingsley and Feigenson[14]. Valinomycin was dissolved in chloroform together with the phospholipid, and solvent removed under high vacuum. Small bilayer vesicles were prepared by sonication in ^2H_2O, or in a NaSCN solution in ^2H_2O having ionic activity of 2.25 M. Alkali cation titrations were performed by adding the thiocyanate salts to the vesicle samples. The exclusive location of the valinomycin within the bilayer and not in the water was determined by chromatography with Sepharose 4B. The valinomycin profile followed the phospholipid profile in both 50 mM NaHPO$_4$ buffer, *p*H 7, and in 3 M KSCN.

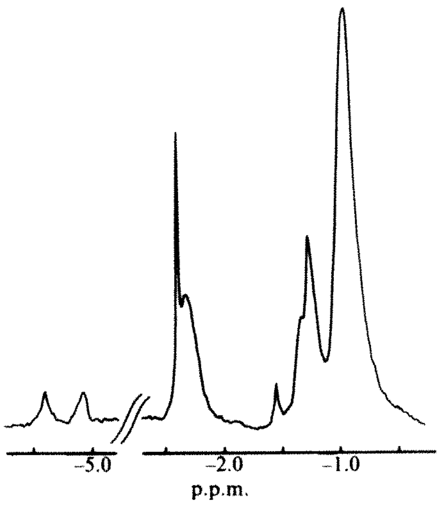

Fig. 1 360-MHz proton magnetic resonance spectrum of 3.1 mol % valinomycin in 11 mg ml^{-1} small sonicated bilayer vesicles of DMPC-d$_{72}$ with 50 mM NaHPO$_4$, *p*H 7, at 50 °C. Transients (48) were collected and a sensitivity enhancement corresponding to a 5-Hz linebroadening was applied. The peak assignments are as follows: −5.39 p.p.m., L-Lac $C_\alpha H$; −5.07 p.p.m., D-HyIv $C_\alpha H$; −2.38 p.p.m., CH_3 from trace tosylate impurity; −2.32 p.p.m., $C_\beta H$ from valinomycin plus residual lipid acyl $C^2_\alpha HH$; −1.55 p.p.m., residual lipid acyl $C^2_\beta HH$; −1.33 p.p.m., L-Lac CH_3; −1.27 p.p.m., residual lipid acyl $(C^2HH)_n$; −0.93 p.p.m., D-HyIv and D,L-Val CH_3.

Resonances from L-Lac and D-HyIv $C_\alpha H$ and CH_3, from D, L-Val CH_3 and from D, L-Val and D-HyIv $C_\beta H$ groups are well-resolved at 360 MHz for valinomycin incorporated into DMPC-d$_{72}$ vesicles in the absence of alkali cations (Fig. 1). Assignments are according to Ohnishi and Urry[5]. Chemical shifts are given in Table 1. The linewidths of 20–40 Hz for the valinomycin resonances, similar to the linewidths for the phospholipids or for other small molecules incorporated into small bilayer vesicles[15], imply considerable local molecular motion. The chemical shift values observed and the temperature dependence of these shifts are close to the values observed in non-polar organic solvents (see Table 1 and refs 8, 16). Spin–spin splitting could not be measured because of the linewidths of 20–40 Hz. This is unfortunate, as the Karplus-type relationship between the coupling constants and the molecular conformation is well-established for many peptides and for valinomycin in particular[8]. Instead, we used the empirical relationship between the observed chemical shifts for valinomycin and its molecular conformation[7,8,16]. Thus, the conformation of uncomplexed valinomycin in bilayer vesicles is the 'A$_2$ bracelet', with quasi-axial carbonyl groups, previously described for valinomycin in cyclohexane and in chloroform[8]. Because of this assignment of the conformation of uncomplexed valinomycin, it is reasonable

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Table 1 Proton chemical shifts for valinomycin and its alkali cation complex at 50 °C

		Chemical shift				
Proton	Residue	Vesicles, uncomplexed	Vesicles, Rb^+ complex*	Cyclohexane, uncomplexed	Dimethysulphoxide, uncomplexed	Chloroform, K^+ complex†
$C_\alpha H$	L-Lac	5.34	5.04	5.31	5.09	4.90
	D-Hylv	5.04	4.61	5.03	4.82	4.55
	D-Val	4.16		4.03	4.26	3.74
	L-Val	4.03	3.72	3.91	4.36	3.85
$C_\beta H$	D, L-Val, D-Hylv	2.32	2.22	2.34	2.18	2.16
CH_3	L-Lac	1.36	1.55	1.36	1.28	1.51
CH_3	D, L-Val, D-Hylv	0.96	0.96	1.00	0.91	1.02

* Extrapolated values.
† Data from ref. 8.

to infer that the location of uncomplexed valinomycin is in a largely non-polar environment. Support for the inference that the predominant location of uncomplexed valinomycin is in the hydrocarbon region of the bilayer must await further investigation.

The chemical shifts of the L-Lac, L-Val, and D-Hylv $C_\alpha H$ and the L-Lac CH_3 can be followed up to high concentrations of added alkali salts, thereby describing a complete ion binding isotherm. The ion binding curves for Rb^+ are shown in Fig. 2. Similar results were obtained for K^+. Addition of up to 3M NaSCN caused no significant chemical shifts, in accordance with the extremely weak binding by valinomycin for this ion in aqueous systems[9,17]. The values for the limiting chemical shifts of L-Lac, L-Val, and D-Hylv $C_\alpha H$ and of L-Lac CH_3 resonances at high concentrations of K^+ or Rb^+ are very similar to the shifts observed in a variety of organic solvents for the fully complexed ionophore[8]. Thus we conclude that the solvent and the bilayer conformations of the fully alkali cation-complexed valinomycin are both of the 'bracelet' type, with carbonyl groups directed inward.

During the course of these titrations each resonance remains as a single peak, up to a shift of as much as 150 Hz for the D-Hylv $C_\alpha H$. Thus, complexed and uncomplexed valinomycin are in fast exchange on this time scale. Any steps in the mechanism of K^+ or Rb^+ binding or release by valinomycin must be significantly faster than $150\ s^{-1}$. Fast exchange for cation complexation has previously been measured for valinomycin in black lipid membranes[18]. This rate must apply to the transfer of the monovalent cations between water and valinomycin, a necessary step in the association. If the ion binding mechanism should also involve a change in the location of the uncomplexed valinomycin, for example from a position in the bilayer interior to a position near the aqueous interface, then this process too must be fast on this time scale. Note that for valinomycin in organic solvents the exchange rate between complexed and uncomplexed forms is slow on this time scale[19].

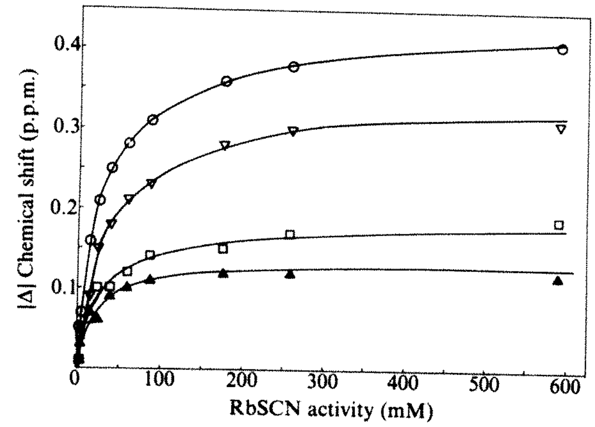

Fig. 2 Rb^+ binding curves for 3.1 mol % valinomycin in DMPC-d_{72} vesicles at 50 °C. The change in chemical shift is plotted against the activity of RbSCN. The activity coefficients used were those for KSCN (ref. 22). ○, D-Hylv $C_\alpha H$; ▽, L-Val $C_\alpha H$; □, L-Lac $C_\alpha H$; ▲, L-Lac CH_3. The sample contained the appropriate concentrations of NaSCN to keep the ionic activity constant throughout the titration.

The titration curves shown in Fig. 2 are hyperbolic, as would be expected for 1/1 binding. Thus this experiment shows no evidence of the formation of complexes of other stoichiometries[20]. When titration of valinomycin is performed by simply adding Rb^+ or K^+ salts to the vesicle suspension with no added NaSCN, thus allowing the cation activity to vary from 0.03 to 0.60 M, the observed chemical shifts were significantly smaller over the first half of the titration than are the shifts shown in Fig. 2. Therefore, any positive charge contributed to the vesicle surface by cation–valinomycin complexes must be more than offset by the adsorption of lipophilic thiocyanate anions in the experiments shown[21].

In conclusion, we have presented the first ^{1}HNMR studies of the conformations of valinomycin within a phospholipid bilayer. We find that uncomplexed valinomycin has a similar conformation in the bilayer and in non-polar organic solvents. Thus, the uncomplexed valinomycin molecule could be located predominantly in the interior of the bilayer. This conformation shifts on K^+ or Rb^+ binding to a structure which is characteristic of fully complexed valinomycin in many different solvents. Complexed and uncomplexed valinomycin are in fast exchange (rate $> 150\ s^{-1}$) throughought the observed range of alkali cation activities of 0.5–600 mM. Further work with this system is now directed toward determining the location and the dynamics of valinomycin in the phospholipid bilayer.

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Elementary particle theory

R.H. Dalitz

An Introduction to Quarks and Partons. By F.E. Close. Pp.481. (Academic: London, New York and San Francisco, 1979.) £26.40; $54.50.

EVEN a decade ago, it was apparent that the proton and neutron, previously regarded as elementary constituents of the atomic nuclei, were actually composite. Very many excited states were known for them and many also for the hyperons and the mesons. The quantum numbers of these excited hadronic states formed definite patterns, recognised to correspond to a 'molecular model' for the hadrons, each baryon consisting of three of the quarks hypothesised by Gell-Mann and Zweig some years before, each meson consisting of a quark and an antiquark. Each pattern corresponded to a definite excitation of their internal spin and orbital motions. Independently, the data on deep-inelastic electron–nucleon scattering had shown rather directly that nucleons contained point-like particles, which Feynman termed 'partons'. With later experiments on deep-inelastic $\nu \rightarrow \mu^-$ and $\bar{\nu} \rightarrow \mu^+$ reactions on nucleons, these data showed that partons and quarks appeared to be identical, having the same spin, baryon number and charge values ($\frac{2}{3}$ and $-\frac{1}{3}$ times the proton charge). This quark–parton picture of the hadrons is now generally accepted.

The quarks are 'point-like' for distances down to 10^{-16} cm, at least, so that they are our present-day candidates for the title 'elementary particles'. However, quarks come in a number of kinds, with differing attributes, known as 'flavours'. There are $N = 5$ flavours established today, the corresponding quarks being labelled u,d,s,c and b, in order of mass, and the discovery of further flavours is expected. The experiments also showed that nucleons contain neutral objects; these are now believed to be the quanta of a 'gluon field' which binds the quarks and antiquarks into the hadron. Finally, in order to give the patterns observed, the Pauli principle required that each quark have three possible states, each now labelled by a colour, and each indistinguishable through the hadronic, electromagnetic and weak interactions we know. This corresponds to an exact 'colour symmetry' and we now believe that the gluon field may be the gauge field for 'colour', just as the photon field is the gauge field for charge. This gauge theory is now known as quantum chromodynamics (QCD). It is a tightly constrained theory, almost unique in form, and it is the theory at the centre of attention today.

Following QCD, elementary particle theory is now undergoing rapid changes and our views today differ greatly from those when this book was finalised. Nevertheless, the book will be much welcomed, especially by students, for it is the only recent introduction available at book length. It does not proceed to expound the subject deductively, from basic principles, but is better described as a discursive review surveying the state of the art in this field of physics in mid-1978. It tends to give results without derivation, although then analysing the result to give some intuitive understanding of its origin and meaning, a procedure having great value for any isolated reader.

Following a brief introduction to set the scene, the book consists of three parts. The first part begins with SU(N) symmetries for N quark flavours, initially for $N = 2$ and then for $N = 3$. This SU(3) symmetry is then extended to an SU(6) symmetry in the space $SU(3) \times SU(2)_\delta$, where δ denotes Pauli spin. In this framework the quark model classifies hadrons according to the product group $SU(6) \times O(3)$. Since Pauli spin is nonrelativistic, this classification cannot refer to a fundamental symmetry; its use today is motivated only by the quark model and is for convenience, allowing the systematic procedures of group theory to be used for economic calculation and tabulation. In general, the observed hadrons correspond to mixed representations of $SU(6) \times O(3)$. The matrix-elements, for the most important transitions, are shown to correspond to the group $SU(6)_W$, so its algebra is discussed in considerable detail, making the usual distinction between SU(6) for currents and SU(6) for hadron constituents and leading to the general Melosh expression for electromagnetic transition amplitudes. Various selection rules are noted, compared with the data and analysed to show how they work. For hadronic decay processes, the transition 'Vacuum $\rightarrow$ $^3P_0(\bar{q}q)$' is introduced, and the resulting amplitudes are related with $SU(6)_W$. Some review is given of attempts to include relativistic effects in quark-model calculations.

The second part is devoted to partons and their phenomenology. The notion of levels of scaling is introduced, and traced (for inelastic electron scattering) from the nuclear to the quark–parton level. The parton distributions in nucleons are deduced from the various leptonic deep-inelastic reaction data and discussed in considerable detail, bringing out their connection with simple field theories. There are brief discussions of scale invariance in electron–positron annihilation at high energies, of neutral currents appropriate to the unified electromagnetic-weak interaction, and of the observed scaling violations, now believed to reflect asymptotic freedom for the underlying field theory. The parton model is extended by introducing quark fragmentation functions, used to analyse and predict hadron spectra and their angular dependence in various high-energy reactions. This leads on to a discussion of large momentum-transfer phenomena in hadron–hadron collisions and to quark counting rules for their asymptotic forms. Specific models such as 'Drell-Yan' and 'constituent-interchange', are introduced and compared with the data.

The third part first sketches briefly the electromagnetic-weak and QCD gauge theories, analysing the qualitative features of the one-gluon-exchange potential between quarks. The major topic is the charmed quark c and its role in the 'new particles', such as the ψ/J and ψ' mesons discovered in 1974, and the D and F mesons established subsequently. Quite a detailed discussion is given of the bound states of Charmonium (c-$\bar{c}$), the 'hydrogen atom' of the 'new particle' physics; all of its properties are well accounted for by QCD, assuming that this can be shown to imply confinement. The heaviness of the c quark (about 1.5 proton masses) justifies a nonrelativistic Schrodinger treatment and the hyperfine structure of the spectrum arises from the one-gluon-exchange potential. Electric dipole γ-transitions occur between its S and P states with rates in accord with these Schrodinger calculations. The long lifetimes of the ψ/J and ψ' states are due to the asymptotic freedom of QCD. The one-gluon-exchange potential and the quark masses are then used to account for all of the mass splittings between and within the various charge multiplets observed within particular $SU(6) \times O(3)$ mesonic and baryonic supermultiplets. There are also chapters dealing with the 'bag model' for hadrons and the possibility of bound multiquark systems. The former is entitled "Quarks confined to a sphere"; despite its very general formulation, the bag model program has been implemented to date only for the case with a rigid spherical bag. The book closes with gauge theories for 'grand unification', where quarks and leptons are assigned to common multiplets and the lepto-quark gauge fields are extraordinarily heavy, with masses of the order of 10^{-10}g. These theories mostly imply that the proton should decay spontaneously (for example, to $e^+ + \pi^0$) and have led to a number of experimental proposals to push up the present limit ($\gtrsim 10^{30}$ years) on the proton lifetime.

This is a book for the quark-model practitioner. It gives all the prescriptions and rules; they are analysed thoroughly but are not always derived (nor even necessarily all consistent). It says something about every idea current today, although some in much more detail than others. The book is somewhat uneven in its treatment, being strongest and most detailed in the areas where the author's own contributions lie (photo-excitation of excited baryons and polarised lepton–hadron interactions). It also has a somewhat dated air, since our picture of elementary particle physics has been changing rapidly. It is now a general belief that the quarks (u,d) are almost massless, whereas most hadron spectroscopy still assumes non-relativistic motion for the quarks in hadrons. The SU(N) symmetries discussed here are now considered accidental, the true symmetry being the flavour independence of quark–gluon interactions, violated only by the quark mass values. Little is said about 'confinement', the central unresolved question for QCD; indeed, the book says rather little about QCD, beyond implications of the one-gluon-exchange potentials. The book has come too soon to include the recent developments in baryon spectroscopy due to Isgur and Karl, which have brought much order concerning Λ^* and Σ^* states. It would have greater value for students today if it had more about the fundamentals and applications of gauge theories, especially for QCD, since the book would then have appeared forward-looking; its present emphasis is on theoretical aspects from the past, which may be expected to change in the near future. But this is perhaps the fate of any book in this field of physics, where exploration is moving fast; it is easy and unfair to make this judgement in retrospect. There is no doubt that every physics library should possess this book, for there is much to be found on its pages which it is not easy to find elsewhere. ☐

R.H. Dalitz is Royal Society Research Professor in the Department of Theoretical Physics at the University of Oxford, UK.

Chondrocytes and their matrix

F.N. Ghadially

Biology of Cartilage Cells. By R.A. Stockwell. Pp.329. (Cambridge University Press: London, New York and Melbourne, 1979.) £25.

THIS book is a welcome addition to the literature on cartilage. It is not a book about the chondrocyte only, as the title might lead one to believe; quite detailed accounts of the morphology and chemistry of various components of the matrix are included as well as other topics such as the nutrition and permeability of cartilage and the nature of the articular surface. All this is successfully accomplished in what the author modestly refers to as a "short book", but the concise style of writing has permitted the inclusion of a volume of factual data which one usually associates with much larger works. The well selected references and a carefully prepared index enhance the value of this work.

It is difficult to fault the text in any major respect. The photomicrographs are rather small in size but of good quality; the same, however, cannot be said of the electron micrographs, which are of a variable quality. In contrast to this the line drawings are superb, and the graphs and tables are well planned and informative.

The book begins (chapter 1) with a brief description of the morphological and chemical differences between various types of cartilage. Chapter 2 gives an adequate account of the ultrastructural morphology of the chondrocyte, the effect of hormones on the chondrocyte and the antigenicity of chondrocytes. Chapter 3 deals with the morphology and chemistry of the matrix components. In this excellent chapter we are treated to quite a detailed account of collagen structure and synthesis, elastic fibres and elastogenesis, and the structure of proteoglycans. However, the morphology of proteoglycans (that is, matrix particles) is barely discernible in the electron micrographs presented. Chapters 4 and 5 deal with the metabolism and nutrition of chondrocytes. Chapters 6 and 7 deal with chrondrocyte differentiation and proliferation. The book ends (chapter 8) with a discourse on degenerative changes, age — associated changes, and a section on calcification in which an up-to-date account of matrix vesicles and their role in cartilage calcification is presented.

In summary, this book provides a brief review of existing knowledge about the chondrocyte and surrounding matrix. It is up to date, well written and packed with factual data. In my opinion this book should be of value not only to research workers as suggested by the author, but also to rheumatologists, orthopaedic surgeons and postgraduate students (residents) interested in cartilage.

F.N. Ghadially is Professor and Joint-Head of Pathology at the University of Saskatchewan, Saskatoon, Saskatchewan.

Primary energy resources

D.O. Hall

Biological Energy Resources. By M. Slesser and C. Lewis. Pp.192. (E. & F. N.Spon: London, 1979.) £8.50.

TODAY about one-seventh (or possibly more) of the world's primary energy is derived from biomass — this is equivalent to 19 million barrels of oil a day (which is twice the Saudi Arabian oil production and equal to the US daily oil use). However, because most of this biomass energy use occurs in the developing countries (and there predominantly in rural areas) and because it seldom enters the official statistics, it has, until recently, been virtually ignored by planners, politicians and aid agencies alike. A combination of the "energy crisis" and desertification problems has focussed the attention of both developing and developed countries on the importance and potential of 'biological energy resources'.

This aptly titled book is a must for anyone interested in the basics of bioenergy and its conversion, and more especially in the energetics and economics of biomass systems. The concepts of energy ratios, net energy yield, net utilisable energy production, and gross energy requirement are well presented, and a number of case studies are thoroughly discussed. It is clearly pointed out that intensification of natural ecosystems for biomass energy requires both capital inputs and better management — but local industry, skills and energy self-reliance can be built up. Such energy systems are applicable to varying extents in diverse conditions around the world and must be tailored to suit local conditions.

The chapter on economics of biomass systems touches on net present value, energy payback times, and economic costings. It considers some specific examples like ethanol production and biogas generation, and compares costs with other energy sources. These costs are changing so rapidly it seems imperative to update one's analyses and comparisons annually to take account of accelerating costs.

It is a good book and very timely. But please could authors and/or publishers try to list references that can be traced — conference proceedings references are useless unless the accurate source is given and organizations who publish booklets, and so on, have their addresses given. In such a rapidly developing field with wide sources of interest it is important to be able to trace the information. ☐

D.O. Hall is Professor of Biology at King's College, University of London, UK.

Grassland ecology

Peter D. Moore

Grassland Ecosystems of the World. (International Biological Programme, 18.) Edited by R.T. Coupland. Pp. 401. (Cambridge University Press: Cambridge, 1979.) £25. *Perspectives in Grassland Ecology: Results and Applications of the US/IBP Grassland Biome Study.* Edited by Norman R. French. Pp. 204. (Springer: New York, Heidelberg and Berlin, 1979.) DM54; $29.70.

SOME thirteen years after the initiation of the International Biological Programme for the collection of information concerning the productivity of the world's biomes, the publication of the accumulated data is gaining momentum. The book books reviewed here are concerned with grassland ecosystems; the first (edited by Coupland) is a condensed comparative synthesis of information from various sites around the world, and the second (edited by French) is a more detailed set of papers from the United States IBP grassland team.

In some areas of the world grassland is believed to represent the climax vegetation type, existing as a response to climate, soil, natural grazing or fire. In other regions, grasslands have developed as a result of human management practices. Often it is difficult to distinguish the two with certainty. In Coupland's book, grasslands are divided initially into climatically determined types and those actually sown and managed by man. Then, within the climatic subdivision, natural and semi-natural grasslands are distinguished — the latter being exploited by man either for hay crops or for grazing.

Each type is dealt with in a separate section subdivided into chapters concerning primary production, consumer organisms, microorganisms, ecosystem synthesis and modelling, nutrient cycling, and the exploitation, management and conservation of grassland systems. This arrangement is particularly valuable, as it permits an easy cross-reference from one section to another. It makes it simple, for example, to check back from tabulations of the efficiency of energy capture in Indian grasslands to equivalent data in a similarly constructed table for prairie systems in the United States.

This facility reflects creditably upon those responsible for the design of the volume and upon the editor, who has ensured a uniformity of expression and presentation which greatly aids those who wish to obtain comparative figures with ease. This must be regarded as the primary function of a book like this one, which seeks to present a global collation of IBP grassland data.

Perhaps a surprising inclusion in the book is a section on croplands, reviewing gramineous agroecosystems. Sadly, this is the least informative section, containing many casual pieces of information in a generally uncoordinated manner. It is largely derived from data emerging from the Polish Agroecological Station at Turew, Poland, but really a wider synthesis of world literature is necessary if this were to be a useful discussion.

Coupland concludes the volume by attempting to make generalisations concerning the structure and production of grasslands. This is a formidable task and tends to take the form of a summary of the range of variation found within grasslands of different types. It is useful mainly in that it identifies those areas of study which need further attention, such as the role of fire in grassland management.

Perspectives in Grassland Ecology, edited by N. French, is more limited in its geographical coverage, being solely concerned with the US grasslands, but it is far more diverse in its structure. It is essentially a series of independant papers concerning grassland studies in North America. Some, such as Lauenroth's paper on grassland primary production, are attempts to review areas of grassland study and to place them in their wider context. Others are concerned with very specific research topics, such as Detling's study of the factors controlling the productivity of the C4 grass ***Bouteloua gracilis*** in mixed grassland in Colorado.

The collection of papers deals with a range of topics, many of which will be of interest to ecologists in general, such as the role of stress in grassland ecology (Dodd and Lauenroth), biomass trophic pyramid structure in grasslands (French, Steinhorst and Swift) and nitrogen input and output (Woodmansee). Finally there is a series of papers concerning ecosystem modelling in grasslands and its value in management studies.

The two books are thus very different in approach. Both supply valuable sources of information and current ideas in the field of grassland ecology. ☐

Peter D. Moore is Senior Lecturer in the Department of Plant Sciences at King's College, University of London, UK.

- *A Handbook of Numerical and Statistical Techniques* by J.H. Pollard (for review, see *Nature*, **270**, 284; 1977) has now been published in paperback by Cambridge University Press at £5.50.
- *Problems of Genetics* by William Bateson, first published in 1913, has now been published with an historical introduction by G. Evelyn Hutchinson and Stan Rachootin, by Yale University Press (New Haven and London) at £12.30 hardback and £3.15 paperback.

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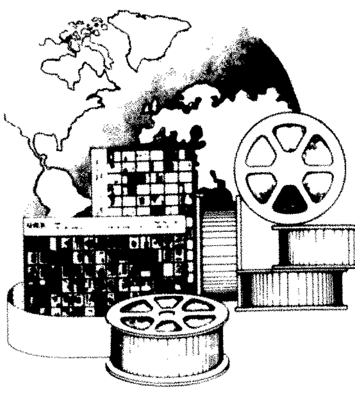

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Particle transport processes

W.A. Wakeham

Transport Theory. By J.J. Duderstadt and W.R. Martin. Pp.613. (Wiley: New York and Chichester, UK, 1979.) £24.

THE study of particle transport processes in a wide variety of fields has been a growing area of activity for a number of years. Inevitably, the developments in the various fields have been largely independent and the underlying similarity of the governing equations for particle transport has been somewhat neglected. This book continues and enhances the modern trend of providing a common basis and methodology for the study of all transport processes and brings together an armoury of techniques for the solution of transport problems.

The fundamental proposition of the book is that transport theory is the mathematical discipline associated with the solution of transport equations. This restricted, but valid, view leads to a volume which is more suited to applied mathematicians than to physicists or engineers. The essential physics of any transport problem is the derivation of a kinetic equation for the problem, the evaluation of the various scattering cross-sections and a statement of the boundary conditions. Of these, only the derivation of kinetic equations is given significant attention in this book. However, the familiar Boltzmann, Fokker–Planck and Vlasov transport equations are obtained with minimum fuss and a short description of the projection operator methods for the derivation of more general transport equations is included. The relationships between the kinetic equations, continuum theory and non-equilibrium statistical mechanics are given a careful and useful exposition.

The elegant mathematical techniques for the solution of transport equations in the integro-differential or integral form, which make up the essence of the book, are well presented. First the simplest transport equations for idealised non-scattering single energy systems are solved and then several more complicated problems are attacked. The necessary mathematical tools are generally developed as required, but a potential reader should already be well versed in integral transforms, boundary value problems and complex variable analysis before attempting the book. Otherwise the treatment of integral equations will be insufficient and the "Child's Primer on the Spectral Theory of Operators", provided in an appendix, will remain obscure. The tool kit for the solution of transport problems is completed by a discussion of numerical methods including finite element techniques and the Monte Carlo simulation methods.

The full battery of mathematical tools is unfortunately applied to very few practical examples in a few problems surprisingly included at the end of every chapter. It is rather as if, when training a mechanic, the theory of a screwdriver was carefully explained but he was never required to use the device to undo a screw. As a practical scientist I found this aspect of the book disappointing since the fields to which transport theory can be applied range from the important subject of neutron distributions in nuclear reactors through light propagation in stellar atmospheres to vehicular traffic flow.

However, the book is written with sufficient humour and insight to lift it above the level of a mere compendium of mathematical methods. In any event, no scientist interested in more than a phenomenological description of particle transport should be without this volume.

W.A. Wakeham is a Lecturer in the Chemical Physics of Transport Processes in the Department of Chemical Engineering, Imperial College, London, UK.

Signal processing

P.W. Hawkes

An Introduction to Statistical Signal Processing, with Applications. By M.D. Srinath and P.K. Rajasekaran. Pp.499. (Wiley: New York and Chichester, UK, 1979.) £18.30. *Digital Signal Processing and Control and Estimation Theory: Points of Tangency, Areas of Intersection, and Parallel Directions.* By A.S. Willsky. Pp.256. (MIT Press: Cambridge, Massachusetts, and London, 1979.) $22.50; £13.50.

THERE are already numerous introductory textbooks on signal processing but the temptation to turn their lecture notes into a textbook continues to be found irresistible by some university lectures. Furthermore, in such a subject, which is expanding into new areas, there is always some fresh application to mention that was not covered in earlier texts. The first of these two volumes is just such a book; it arose from various courses of lectures in the Southern Methodist University (Dallas) and two Indian institutes, and covers detection and estimation theory in commucations, radar, pattern recognition and system identification. It is a competent if uninspired work, clearly written for the most part though rather pedestrian in tone, and contains many worked examples, for which students will be grateful. Although it is described by the authors as a graduate text, much of the material is taught at undergraduate level in British universities, and for this audience, the book is probably too detailed: lecturers and students alike will probably find that their preferred text on signal processing, supplemented by a volume on two-dimensional questions in general (pattern recognition, image processing and the like), is more suitable.

Willsky's book is a very different matter. The author is a control theorist who was struck by the fact that work in digital processing and in control and estimation theory has a great deal in common, as well as certain clear differences that are themselves illuminating. There is no doubt that these two groups of subjects have much to offer one another and the author explores a range of topics from both points of view.

This kind of book is in the best sense a piece of self-indulgence on the part of the author, a leisurely examination of difficult problems and unsolved questions by a wide-ranging mind, not under any immediate compulsion to provide solutions. The result is an enjoyable and stimulating book, easy to read despite the advanced nature of the contents although the reader is expected to be familiar with the vocabulary, verbal and mathematical, of the subject. There are five main chapters, concerned with (1) the analysis of stability, (2) parameter identification and related topics, (3) synthesis, realization and implementation, (4) multiparameter systems, distributed processes and random fields, and (5) nonlinear system analysis.

The flavour of the text is well illustrated by the opening remarks of the chapter on multiparameter systems, which is much concerned with image processing: "This area is rich in both potential applications and challenging theoretical problems . . . How does one process distributed data efficiently? What properties do recursive techniques have when the recursion is in more than one dimension? Do causality and state make any sense here? What about stability? What are the tools for analyzing stochastic processes? How do we "predict" when time is not the independent variable? What role do recursive estimation techniques play? What are recursive estimation techniques? Which concepts concerning signals and systems in one independent variable carry over to the multiparameter case? Which do not, and why don't they?". Altogether an interesting and provocative book.

P. W. Hawkes is Maître de Recherche in the Laboratoire d'Optique Electronique du CNRS, Toulouse, France.

nature

24 January 1980

Curing Europe's biological death-wish

LAST WEEK an apparently European firm, Biogen, incorporated in Luxembourg and with a laboratory in Geneva, announced that it has successfully engineered *Escherichia coli* to produce interferon — currently touted both as an anti-viral and as an anti-cancer agent, after some small trials have shown remissions; larger trials are on the way. So does Europe rejoice at its technological breakthrough?

Unfortunately not, for Biogen stockholdings are held almost exclusively in North America. The major exceptions are the seven European scientists involved; with two Americans, the scientists hold one third of the stock. The largest individual stockholder is International Nickel, a Canadian-based mineral company, with the drug firm Schering-Plough — which has bought the international licence to exploit the Biogen creation — in second place. A handful of other bodies have holdings of under 10%. Hence the remark of Charles Weissman, who co-engineered the interferon-producing bug, quoted in this issue (page 323), that it was a shame that European industry had not taken greater interest in Biogen. Apparently those who were forming the company approached a number of firms in Europe, particulary in Switzerland and West Germany — but none was interested in a share in the business.

The fault, then, was doubly Europe's: first, it was a fault not to attempt to draw on its own potential bioengineers through its own 'Biogen' — noone was adventurous enough to set it up, though many had the idea; and second, it was a fault to ignore a ready-made opportunity — Biogen itself — when it was offered.

Furthermore in the UK a number of scientists have been attempting for three years to interest venture capital and the government in a British contract research organisation, akin to Biogen, to no avail. Some who were active in that attempt are now members of Biogen. The National Research Development Corporation, which might be expected to take a lead in such matters, seems content merely to exploit. Whereas four of six major revenue-earners are biotechnical, its past and present biological development projects are outnumbered by the non-biological 10:1.

Neither do European politicians have much to praise each other about. When it began to be possible to make high fructose syrup economically from maize starch using immobilised enzymes, the Council of Ministers of the European Community — the highest and most political authority — slapped a punitive levy on the process, killing its development (which was already underway in Britain), in the name of protecting beet growers.

No wonder that a draft version of the forthcoming Royal Society report on biotechnology recommends that Europe puts its house in order. Biotechnology must not invite penal levies, says the draft, merely because Community policy is in the grip of interests which "resist the cheaper and better products of this new technology, particularly where these threaten to perturb existing patterns of agriculture". Common Agricultural Policy legislation which threatens biotechnology must be revised; and arrangements reached whereby agricultural surpluses can be turned to good use as biotechnical feedstocks.

In this and other ways the report is a strong blast of good sense which the British government — and the Community — would do well to respect. Recession, when individual firms are without the funds to invest, is exactly the moment when governments can move in legitimately to pick up the bill for long-term, strategic research. This also happens to be exactly the moment when technology is finding a new lease of life, through microcircuits and now biotechnology. The nations which will dominate the economics of the 21st century will be the ones that have invested in the new ideas — the ones that have the new processes underway and the new talent in their industries. Engineered bugs and microchips will be the coalfields of the next century: and they are not to be found, but created.

Two great opportunities are shortly to be forthcoming in biotechnology: the £16 million research programme proposed for the EEC which the European Commission should not foolhardily reject; and the recommendations of what will, we hope, be a not too diluted version of the draft report we describe this week (page 324). Europe and the UK must embrace both of them. □

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United States

Regulators loosen up on carcinogen standards

Labour unions and the chemical industry have locked the US Occupational Safety and Health Administration in a dispute over translating carcinogenicity data into appropriate regulatory controls. **David Dickson** reports

LAST week Dr Eula Bingham, head of the US Department of Labour's Occupational Safety and Health Administration (OSHA), unveiled the final version of the agency's new scheme to identify and regulate carcinogens in the industrial workplace.

The scheme was orginally proposed more than two years ago as a way of streamlining the regulation of cancer-causing substances. The intention was to set out broad criteria for deciding how to deal with certain types of data in determining whether a substance is carcinogenic. Mr Ray Marshall, Secretary for Labour, has hailed it as a "landmark policy" and a "tremendous step forward" in protecting workers from cancer.

But in attempting to find a position that fulfils OSHA's congressional mandate while also minimising its vulnerability to the inevitable legal challenges, the agency has loosened some of its original proposals, adding the criticism of labour unions to that of the chemical industry.

The industry, while pleased with some of the new flexibility, remains unhappy with the scheme. And the American Industrial Health Council (AIHC) , representing a number of leading chemical companies such as Dow Chemical and Monsanto, has already asked a federal appeal court to review the regulations, claiming that they "lack scientific validity" and impose an unreasonable economic burden.

But OSHA is now facing opposition from one of its previous supporters, the American Federation of Labour — Conference of Industrial Organisations (AFL-CIO), which has filed its own suit in the District of Columbia, claiming that by relaxing some "cornerstone" provisions the agency has weakened the scheme's effectiveness.

In broad outline, OSHA's new policy for regulating potential suspected carcinogens (see box for definition) is comparable with that first suggested in 1977. In particular, the agency has stuck to its proposal to place suspected carcinogens in two principal categories. The first contains substances for which there is good scientific evidence of carcinogenicity; exposure to such substances is to be reduced to lowest feasible levels, or eliminated if substitutes can be found. The second category contains substances for which there is "suggestive" evidence of carcinogenicity.

As previously, OSHA states that, in evaluating claims of carcinogenicity, it will not accept a 'threshold' hypothesis, and will still give greater weight to positive animal studies showing evidence of carcinogenicity than to 'non-positive' human studies showing no such evidence.

However the agency has shifted from laying down strict, formal criteria for making such assessments, to relying more on the judgment of scientists, in particular through the use of scientific review panels.

Thus several alterations have been made in the criteria used to classify a substance as belonging to category 1. For example, it was originally proposed that a substance could be included in this category if it was shown to be carcinogenic in one animal bioassay, the results of which were subsequently replicated.

OSHA received various objections to this criterion, some claiming that at least two animal tests were necessary, others that a single, well-conducted test should suffice, and that to require replication or confirmation would unreasonably delay regulatory action.

Responding to these criticisms, OSHA has agreed to amend its criteria so that now a substance can meet the definition of a potential term bioassay "where the results are in concordance with some other scientifically evaluated evidence of a potential carcinogenic hazard" or in a single mammalian species "in which the secretary determines the requirement for concordance evidence is not necessary".

A 'potential suspected carcinogen':

Any substance, or combination or mixture of substances, which causes an increased incidence of benign and/or malignant neoplasms in humans or in one or more experimental mammalian species, or a substantial decrease in the latency period between exposure and onset of neoplasms in humans or in one or more experimental mammalian species as the result of any oral, respiratory or dermal exposure, or any other exposure which results in the induction of a tumour at a site other than the site of administration.

A positive result in a short-term test would be:

Positive results in assays for two or more of the following type of effect:

- The induction of DNA damage and/or repair
- Mutagenesis in bacteria, yeast, *Neurospora* or *Drosophila melangaster*
- Mutagensis in mammalian somatic cells
- Mutagenesis in mammalian germinal cells
- Neoplastic transformation of mammalian cells in culture.

Evidence of 'concordance' may include positive results from testing in the same or other species; or positive results in short-term tests (OSHA has reaffirmed its previous position that such tests can be used to confirm, but not to substitute for, bioassay data, claiming much of the criticism of these tests to be "unsubstantiated"); or the induction of tumours at injection or implantation sites (despite protests from industry, OSHA has again refused to distinguish between malignant and non-malignant tumours as evidence).

In contrast to its earlier proposal, OSHA has decided to drop two further classificatory proposals, one covering substances for which there was a suspicion, but no hard evidence, of carcinogenicity, and the other for substances not occuring in US workplaces.

More controversially, the agency has also decided that preliminary classification in category 1 will not, as previously proposed, automatically require the Secretary of Labour to issue an 'emergency temporary standard' requiring companies using the substance in question to reduce exposure to lowest feasible levels, at least until firm regulations have been established.

Rather, OSHA will publish at the beginning of each year a list of potential candidates for each category. Preliminary estimates indicate that there could be about 150 chemicals in category 1 and 350 in category 2. Every six months OSHA will then select about ten substances from each category for priority attention, with the Secretary of Labour having the power to impose emergency standards if he felt that the chemical poses a "grave danger" and that such a standard is needed.

This added flexibility has angered the labour unions, strong supportors of automatic standards for category 1 substances. OSHA points out that it may lack the legal powers to carry out such a policy — and officials add that if OSHA did introduce such a rigid ruling, there might be a reluctance to place substances into this category because of legal challenges.

But the AFL-CIO is disputing this, complaining that by dropping what it calls "indispensable provision", OSHA has removed the "cornerstone" of its cancer policy, in conflict with the requirements of the Occupational Safety and Health Act of 1970 requiring it to take all steps necessary

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to protect the health of US workers.

The chemical industry has, in general, welcomed the new flexibility in the rules — although it still considers them to be too harsh. For example industry rejects OSHA's criteria for accepting epidemiological evidence of non-carcinogenicity.

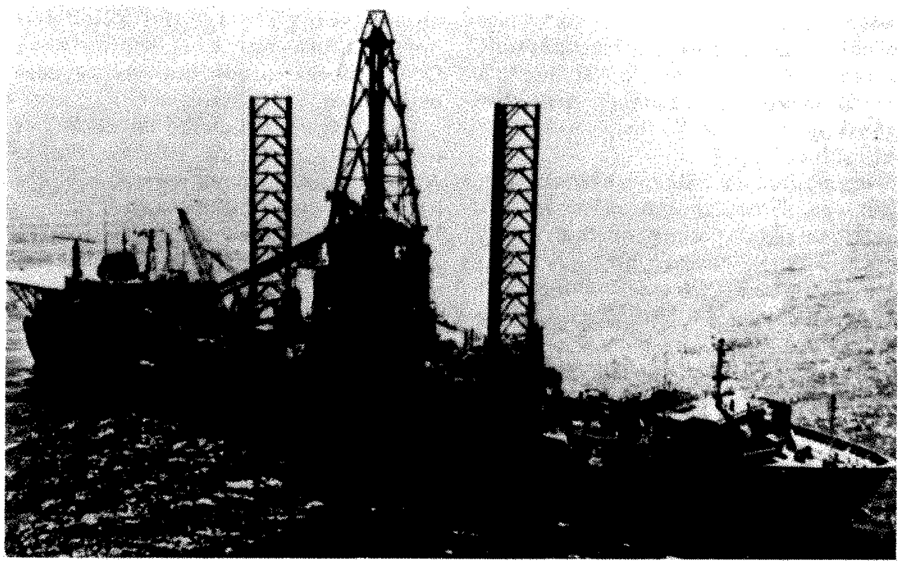
And the industry remains critical of the costs. Dr Bingham, in presenting the new scheme — scheduled to be published this week in the *Federal Register* — said that some studies now showed that the costs of cancer exceeded $15 billion a year.

But she repeated the agency's opposition to using cost-benefit analysis in its rulemaking rather than restricting itself to calculations of economic feasibility. Allegations that OSHA was trying to create a "risk-free" environment, regardless of cost, "conveniently overlook that Congress charged OSHA with protecting workers to the extent feasible" she said.

But the industry is not convinced. Significantly the AIHC has chosen to file a suit requesting federal review of the new regulations with the fifth circuit court of appeals in New Orleans. It was this court which, two years ago, rejected OSHA's proposed new benzene exposure standards on the grounds that the agency had used cost-benefit techniques in reaching its conclusions. The case is now before the Supreme Court for final arbitration. A decision against OSHA could have a devastating effect on OSHA's effectiveness. □

US to announce $700 million ocean-drilling research programme

In a unique experiment in cooperation with private industry, writes **David Dickson**, the US government and leading oil companies are about to announce plans for sharing the costs of a 10-year research programme to investigate the Earth's continent margins



Glomar Explorer: to be made use of in the new programme

WHEN President Carter presents his 1981 budget proposals to the US Congress next Monday, he is expected to include plans for one of the biggest and most ambitious research projects ever sponsored by the National Science Foundation: a 10-year programme to investigate the geology of the ocean floor.

The programme offers potential payoffs both in terms of increasing understanding of the history and dynamics of the Earth's crust, and of assessing the exploitable resources — in particular ore and hydrocarbon deposits — which the crust contains.

And in a unique arrangement largely credited to the President's science adviser, Dr Frank Press, the estimated $700 million costs of the programme are to be shared equally by the federal government and private oil companies.

Final details of the agreements are yet to be reached. But it is understood that at least eight companies have agreed in principle to participate in the scheme. Several others have indicated their interest.

At a later stage, it is also intended that the programme will include the participation of research teams from other countries. Britain, France, West Germany, Japan and the Soviet Union each contribute $1 million to the NSF's current deep-sea mining programmes, and several have expressed interest in participation in the new project, as have Canada, Mexico and Australia.

Speaking at the Woods Hole Oceanographic Institution in Boston, Massachusetts, last week, Dr Press said that the administration intends to make use of the ship Glomar Explorer for the ocean drilling project. Originally built by the Central Intelligence Agency to attempt to retrieve a Soviet submarine from the Pacific floor, the vessel has more recently been used by private corporations for investigating the potential of sea-bed minerals. It is considered almost ideal for the type of operation currently envisaged.

The idea of using the Glomar Explorer for ocean-floor drilling has been actively discussed in the geological research community for several years, especially since interest has focused on the ocean margins, the zone between the continental shelf and the deep ocean basin.

For the past 12 years, the NSF has supported a major deep-sea drilling project from the smaller Glomar Challenger. But although the 'challenger' has produced much important science, leading to increased understanding of plate tectonics and other geological processes, it has been limited in its scope.

In particular, the Challenger lacks the equipment to prevent a blow-out should it hit a pocket of pressurised oil or natural gas during experimental drilling. This has restricted its ability to investigate the continental margins, since it is in these regions that such pockets are likely to occur, rather than in the central ocean basin.

To explore such regions, while at the same time minimising the potential danger resulting from a blow-out, drilling will have to be carried out with the aid of a 'riser' — a protective shell surrounding the drill — as well as other appropriate preventitive equipment.

The general desirability of a large-scale programme of ocean drilling has been supported by various groups who have studied the idea, in particular by a special committee of the Joint Oceanographic Institutions for Deep Earth Sampling (JOIDES) whose report on the matter was issued in 1977.

Further reports prepared for the NSF endorsed the scientific importance of a deep-sea drilling project, designed to reach the ocean floor at a depth up to 4,000 metres and drill 7,000 metres below that, and indicated that such a programme was technically feasible, with the use of the Glomar Explorer as the most promising platform to drill from.

In order to study the overall importance of the programme in the context of national scientific priorities — a move requested by Congress in reviewing the Foundation's 1979 budget request — the NSF established a top-level committee under Dr Guyford Stever which recommended last summer that ocean

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margin drilling "should be given high priority consideration for approval" in the NSF's 1981 budget.

The report also supported moves to gain support from private corporations in the US. This strategy has been actively canvassed by the Office of Science and Technology Policy, which Dr Press directs. And although corporate involvement had been objected to by some scientists on the grounds that it could steer the project away from basic research priorities, it soon became clear that the financial climate would not support the project on federal funds alone.

Last autumn both Dr Press and Dr Richard Atkinson, Director of the NSF, spent considerable time and effort talking to the heads of major US oil companies soliciting their support. The reactions were generally favourable, and in response to a formal approach, a number have now agreed in principle to help match the federal funding.

Negotiations have at times been delicate, given in particular the price sector's traditional reluctance to cooperate directly with the federal government, rather than on a contract basis. And various measures have been discussed to make the deal more attractive.

For example, it is expected that companies will not, at this stage, be required to commit themselves for the full ten years, but will have the option of pulling out after one year or after five years. It is also likely that the first location chosen for investigation will be off the North Atlantic coast of the US, an area of interest to the petroleum companies.

Details of how the scientific programme will be organised are also under discussion. One likely arrangement is that the scientific efforts will be coordinated by the Joint Oceanographic Institutions Inc, a consortium of nine institutions involved in oceanographic research. There would also be three policy review boards, one established by the National Academy of Sciences, one by the Department of Defense, and one by various private institutions and corporations.

Total funding request for the next financial year is expected to be about $20 million, half coming from the NSF — whose total deep-sea drilling budget would increase from $18 million to about $32 million, and half from the oil companies.

So far, despite some reservations about the corporate involvement, the reaction of most geologists to the scientific opportunities has been enthusiastic. Congressional reaction, however, has been more mixed.

Representative Edward Boland, for example, Chairman of the House Appropriations Committee responsible for the NSF budget, has already written to President Carter urging full support for the project. But others are more sceptical, one congressional staff member pointing out that the cost per hole will be at least an order of magnitude greater than with the Challenger.

But the administration is convinced that it is sufficiently important to merit substantial support. "This will be an important scientific venture with possible implications for future resource discovery" said Dr Press. "We believe it is also a good example of government-industry cooperation, the kind of cooperation that we must see more of, in more fields, in the coming years". ☐

Laser fusion project to be halted?

PRESIDENT Carter is expected to recommend to Congress next week a halt to construction work on the $200 million Nova laser fusion project, which began last year at the University of California's Lawrence Livermore Laboratory as a successor to the Shiva system first operated in 1978.

Administration officials in Washington confirmed last week a story appearing in the *Washington Post* which claimed that the Office of Management and Budget had recommended that funding for Nova be dropped from the President's budget request for the fiscal year 1981.

The agency is said to have been concerned partly about the cost of the project, and partly about growing uncertainties about whether glass laser technology can meet the efficiency of a commercially viable source of power.

Congress authorised $23 million for Nova in 1979 — and a further $56 million for 1980. The foundations for the new machine are currently being laid near Shiva.

A number of recent reports, however, have indicated that the project should be slowed until more is known of the physics of laser/pellet interaction, and of the engineering problems involved.

Officials at Lawrence Livermore are making no public comment until the final budget is published next week. But if funding for Nova is dropped, this is likely to be firmly resisted by the armed services committees in both houses of Congress, which support laser fusion research on account of its potential military applications.

Soviet Union

Science planners and the Press tackle the 'two cultures'

SOVIET science planners have taken up the "two cultures" controversy of CP Snow. A new Press "discussion", launched last week by the prestigious *Literatunaya Gazeta* will deal with the falling popularity of the applied sciences and its possible effect on the Soviet economy.

The new campaign, which will include expert articles and readers comments, is entitled "Applied science; prestige and people". It is intended to look into the factors hindering the implementation in production practice of new scientific results. This is not a new theme — the slow rate of implementation of R&D into industry has for some years been a recurrent complaint of planners and Party leaders alike.

Recently, however, the problem has been seen in a somewhat wider context. In December 1979, Moscow radio put out a Press-round up on the dirft of Soviet school-leavers away from science. Instead of the "infatuation" with science and technology, which had characterised university applications for the past two decades, there was now, said the commentator, a significant increase in applications for medicine, economics and teacher-training and journalism courses. So long as this drift from science reflected a genuine response of young people to "spiritual values", he concluded, it was, on the whole, a good thing.

Literaturnaya Gazeta, however, is less approving of the drift. In the course of creation of a scientific society, it observes, a "certain stereotype mode of thinking" has grown up, which considers that "applied research in the interests of this or that branch" is "second-rank and non-prestigious". The two opening articles of this discussion set out to contradict this assumption. The first, by A Savelev, a sector-chief of the Central Scientific Research Institute of the Sewing Industry, cites C P Snow to the effect that many "pure" scientists fail to grasp the fact that many engineering problems are in no way inferior as regards explicitness and strictness to those with which they (the scientists) are concerned. And Boris Smagin, a leading science writer says that many Soviet scientists including Vernadskii, Bardin and Kurchatov "always placed the interests of science on the same level as the interests of the national economy".

The problem of prestige, however, is, as Smagin admits, a "mysterious" one. More precisely, in the words of an editorial footnote, the "prestige of the profession of applied-scientist is a social-psychological concept." The Press discussion of the role of prestige, explain the editors, is intended to focus attention on this. **Vera Rich**

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Switzerland

Engineered *E. coli* produce interferon

THE announcement, last week, by Professor Charles Weissmann of the University of Zurich that his laboratory, on behalf of Biogen, had managed to recover biologically active interferon from bacteria containing human interferon DNA, establishes Biogen as a company that means business. Although the yield of interferon so far obtained from the genetically engineered bacteria is far too low for commercial production, Professor Weissmann thinks that "if things work out as hoped, we should have a high yielding strain within six to twelve months and we could then move relatively fast".

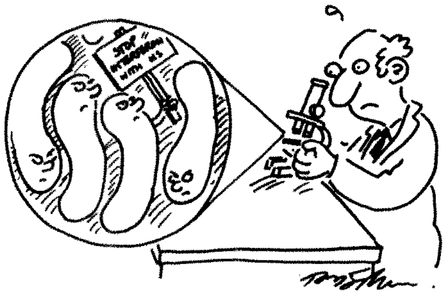
Until recently interferon has only been available in very small quantities with a purity of less than 1%. It has been produced by laboriously purifying human lymphocytes taken from blood donated for transfusion in Finland. A number of very small scale clinical trials have suggested that interferon is effective not only as an antiviral agent but also in the treatment of neoplastic disease. Because of the success of the initial trials there is now a tremendous demand for sufficient interferon to set up bigger trials.

The man behind the Finnish programme is Dr Kari Cantell. He provided Biogen with the human lymphocytes from which its genetic engineering approach started. The first step in that approach was to prepare complementary single stranded DNA from a crude mixture of messenger RNA extracted from virus-stimulated human lymphocytes.

Since there was no way to isolate the sequences corresponding to the interferon gene from the DNA, it was necessary to clone the DNA at random (using the plasmid pBR322 as vector and *Escherichia coli* as the bacterial host) and then to screen the resultant bacteria for any that contained the interferon gene.

Five thousand bacterial clones were screened by extracting their plasmid DNA. The plasmids containing interferon DNA were determined from their ability to direct the production of biologically active interferon when injected into oocytes of a frog. Having identified individual bacterial clones containing interferon genes, Professor Weissmann's team established that the bacteria were producing interferon with much the same biological and chemical characteristics as authentic human lymphocyte interferon.

It remains to be seen whether the bacteria can be further manipulated to increase their yield of interferon, currently running at one thousand fold less than that produced from an equivalent culture volume of human lymphocytes. Professor Weissmann is confident that that can be done. He is somewhat more concerned about the potential clinical activity of bacterially produced interferon, since it is unlikely that bacteria can add the carbohydrate chains that are naturally present on the protein molecule. Current indications, however, are that this will not matter clinically.



If the carbohydrate groups do turn out to improve clinical activity, genetic engineering will probably be a less profitable route to interferon than the alternative approach of cell culture, which is already producing enough interferon for some clinical trials. For example, the Wellcome Research Laboratory in Beckenham, UK already extracts interferon from virus-transformed lymphoblastoid cells in sufficient quantities to satisfy a UK Medical Research Council sponsored trial in myelomatosis. Many, perhaps dozens, of other laboratories are undertaking a similar approach although some of them, such as Searle Laboratories in High Wycombe, UK, use fibroblasts rather than lymphoblastoid cells.

Biogen is not alone in its genetic engineering approach to interferon. Amongst other industrial concerns, some of them with an undeclared stake in the business, Searle Laboratories and the Dupont Chemical Co (in Wilmington, Delaware) are thought to be particularly close on Biogen's tail. Dupont in collaboration with the California Institute of Technology has recently sequenced one end of interferon. They will now probably synthesise the complementary DNA sequence and use it to fish out bacterial clones that contain interferon genes.

What must already concern Biogen's industrial competitors is the patents that Schering-Plough have applied for on behalf of Biogen in which they hold 15% of the shares. Professor Weissmann says that the patents are connected with the plasmid rather than the process and no one knows whether they will be granted. In any case, most of the eventual profits that accrue from interferon or any other Biogen product will finish up in the US, a fact which does not please Professor Wiessmann, himself a shareholder.

It is a great pity, he told *Nature*, that none of the Swiss or German companies that were originally approached was willing to take a major share in Biogen. The more the pity because several eminent European scientists are attached to the company and because its own laboratories are in Geneva. These are already occupied by the first recruits and a director of research is being sought. The interferon work has been carried out during the last 18 months in Professor Weissmann's own laboratory but was paid for by Biogen.

Peter Newmark

United States

Registration proposed for private DNA research

A bill that would require companies to register details of their research and production activitities involving the use of recombinant DNA techniques is to be introduced into the US Senate by Senator Adlai Stevenson, chairman of the Science, Technology and Space Subcommittee.

The proposed bill, which Senator Stevenson refers to as a "reasonable alternative" to comprehensive regulation, would cover individuals and institutions not presently required to observe the National Institutes of Health's safety guidelines. The Secretary of Health, Education and Welfare would have to be notified of all research, development and production activities using these techniques — including the source of the DNA, the type of hostvector system used, and the volume of growth media — with a civil penalty for non-compliance.

The bill also includes procedures to guarantee the protection of trade secrets. And to provide an opportunity to evaluate claims of voluntary compliance with the NIH guidelines, it would also require the secretary of HEW to report annually to Congress on the adequacy of safety precuations reported to have been taken.

Senator Stevenson is proposing to schedule hearings within the near future on the status of current regulation and development of recombinant DNA research. How far the bill goes, however, will depend partly on the support that it gets from Senator Edward Kennedy's Human Resources Subcommittee, which has jurisdiction over medical research — and with the presidential campaign now in full swing, the subcommittee is not expected to give the bill high priority.

David Dickson

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United Kingdom

Biotechnology report urges £10 million programme "to match competitors"

A MAJOR report on biotechnology in Britain — not yet published but made available to *Nature* — calls for a rapid increase in investment to build a competitive industry. It says the "customer-contractor" principle should be scrapped for biotechnology, where the border-lines between basic and applied research are grey. The report is a first draft — though unlikely to be substantially altered — from a seven-man working party set up early last year under Dr Alfred Spinks, a former research director of Imperial Chemical Industries. **Robert Walgate** reviews its hard-hitting and interventionist recommendations.

THE working party was drawn from the Royal Society, the Advisory Board for the Research Councils and the Advisory Committee for Applied Research and Development. It was set up under the previous Labour administration; during its deliberations a Conservative government, committed to non-interference in industrial affairs, has taken office, but this has not restrained its far-reaching proposals.

It says the whole foundation of biotechnological research in Britain should be changed:

"Since 1972, the UK has unlike other countries adopted a very rigorous customer-contractor principle for all its applied R&D funded by government. This approach is well-adapted to short-term or tactical applied work with clear objectives which can be defined by a single customer.

But biotechnology epitomises a field in which scientific knowledge and technical expertise are evolving rapidly, where definite customers have yet to develop in any number; where the research of government departments is divided; where the research will be of interest to a wide range of private industry, public services, etc.; and where the principle need for growth is in strategic applied research much of which will not yield results of immediate commercial benefit for perhaps a decade.

"For such a field the customer-contractor principle has serious shortcomings, and may exacerbate the situation by emphasising an arbitrary and unreal division between fundamental and applied research." Nevertheless the ultimate objective should be to make British industry competitive with the best of its foreign rivals.

Among its recommendations are:

- That the research councils and the ABRC should set up a Joint Committee for Biotechnology to develop a coherent programme of biotechnology research and to coordinate spending of not less than £3million annually (including existing research council initiatives). The Joint Committee would aim to set up new projects in universities and elsewhere, and "should encourage collaboration between experimenters of different disciplines, in different departments and institutions". Applied biologists currently working in medical or agricultural studies should be encouraged to take up biotechnology. A condition might be applied to applicants for grants that they should show evidence of industrial interest in their project.

- That an Interdepartmental Steering Group should be sent up to coordinate the actions of ministries and develop a programme of industrial R&D. The Department of Industry might take a lead in this, setting up cooperative programmes with industry (funded 50:50), research associations, government research establishments, universities, and inventors amounting to £2.5million annually (including existing projects).

- The Steering Group should consider setting up a Support Unit, like the Department of Energy's Energy Technology Support Unit, to do desk studies and build up a list of potential projects for which applications would be sought.

- The Joint Committee and Steering Group should have overlapping membership to avoid a division between basic and applied work, and in any case should review the position in 4-5 years' time.

- There should be no new establishments specifically to pursue research in biotechnology; these can stagnate and exacerbate interdisciplinary divisions. But the Department of Industry should co-sponsor the Centre for Applied Microbiological Research at Porton Down (it is presently sponsored by the Public Health Laboratory Service); CAMR expertise could be well-applied to biotechnology.

- The University Grants Committee and the Committee of Vice-Chancellors and Principals should support the expansion of a limited number of centres of excellence in biotechnology from the best existing in universities. A minimum of 20 new teaching and research post should be established over the next five years with a capital investment of around £2million to provide additional laboratory facilities.

- Urgent provision must be made for training a work-force to match the expected growth of biotechnology; greater interaction should be encouraged between departments and undergraduate courses in the biological, chemical and engineering sciences.

- The Science Research Council's Cooperative Awards In Science and Engineering, which funds research students in joint projects between university and industry, should be extended to the Agricultural and Medical Research Councils; and there should be analogous post-doctoral awards.

"Government should not impede the rapid development of biotechnology by inappropriate or unfounded concern for existing industries or the conjectural hazards apparently presented by some aspects of the new technology."

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Dr Spinks: he chaired the working party.

- The National Research Development Corporation, one of whose roles is to patent and license appropriate inventions made in universities, "should play a more entrepreneurial role in this area, where inventions are often not readily patentable. The NRDC should expand its staff in biotechnology and review existing financial incentive for academic inventors. The NRDC should be at least as concerned with the development of new business as with obtaining and licensing industrial property.

- The NRDC should continue to pursue with the Patents Office the patenting of microorganisms and press for arrangements, as in the US, which do not require the microorganism to be made available until the patent is granted.

- As new research defines the hazards of genetic manipulation, the UK in consultation with others from abroad should modify regulations appropriately. The Genetic Manipulation Advisory Group and the Health and Safety Executive should continue to reduce constraints while maintaining an adequate level of safety and introduce procedures to ease industrial application.

- The National Enterprise Board and the NRDC should investigate the use of public funds to set up a research oriented biotechnology company (akin to the American Biogen and Cetus). £2million a year for five years should be sufficient to establish if it could be profitable, the report suggests.

- The Ministry of Agriculture, Fisheries and Food and the Department of Industry should jointly investigate the use of agricultural products as feedstocks for biotechnology.

- The Department of Health and Social Security should adjust its purchasing policy to encourage small biotechnological firms.

- The Department of the Environment together with the Department of Industry and the Natural Environment Research Council should consider the use of biotechnology for waste disposal and materials recovery.

- If the European Economic Community decides to pursue its research programme in biotechnology, the UK should seek maximum return from it.

- Government should seek fiscal regulation within the EEC which will allow the use of agricultural feedstocks for industrial biotechnology "and not impede the introduction of new processes based on biotechnology". The report here criticises "the strong lobbies of established industry and agriculture which tend to exert a disproportionate weight in resisting the cheaper and better products of new technology, particularly where these threaten to perturb existing patterns of agriculture". There should be assurances that processes "will not invite penal levies . . . We are concerned that this appears to be the present case."

- Addressing itself to the European Community, the report recommends that "all member states should consider the value of biotechnology in transforming agricultural surpluses, and should seek to amend the Common Agricultural Policy legislation that threatens this end". Further, the EEC directive on the control of genetic manipulation is outdated and should be "reconsidered".

- The report "supports" the SRC in a £1.5million capital, £1million annual expenditure on biotechnology; commends the ARC's initiatives in genetic manipulation, and makes a mild and indirect criticism of the MRC: "The MRC and research councils should review their roles in relation to industrial development based on biological processes so that their staff are aware of the desirability of creating, where possible, a national return for their efforts through industrial development."

Despite the case of cephalosporins (antibiotics emerging from MRC research which are now the NRDC's single greatest revenue earner) "the take-up of discoveries by UK industry is not encouraging". Extra facilities might be provided to researchers for applied work in their own laboratories, or a special development unit might be established. However, the MRC was not set up with industrial development as an objective, and any moves along these lines would require extra resources.

National Collection of Yeast Cultures threatened

THE UK National Collection of Yeast Cultures, one of the most used in Europe, faces closure at the end of this year — unless the Agricultural Research Council can find a meagre £20,000 per annum to support it. "I don't see how biotechnology can carry on without a repository of cultures from which one can select strains" said a yeast microbiologist last week.

The collection has been looking for a home since early 1979, when one of its present co-sponsors, the Brewing Research Foundation, announced that it would withdraw its one-third contribution to the costs of the collection from January 1981. The major sponsor, the Ministry of Agriculture, Fisheries and Food, also decided to withdraw at the same date. The collection must also move from its present location at the BRF, in Nutfield, Surrey.

All would be well, say microbiologists, if there was some firm promise of new funds and a new place for the collection. But despite detailed recommendations to the Agricultural Research Council — who it is widely believed should support the collection — from the UK Federation for Culture Collections, no decision has been reached on either location or funding.

"Individual collections are not sufficient" said one microbiologist. "We keep our own small collections but we do not have time, competence or staff to do routine testing or distribute samples. It would be very serious if we sent the wrong organism".

The UKFCC has recommended that the collection and its four staff be established at the ARC Food Research Institute in Norwich, on a site where the John Innes Institute and the University of East Anglia are already established.

MAFF has placed the problem firmly with the Advisory Board for the Research Council's, which in turn has passed the problem to the ARC. But it would appear that the ARC is not prepared to fund the collection unless it receives extra finance.

Barbara Kirsop, the curator of the collection, told *Nature* that the collection has been in financial difficulty for four years. A number of agencies picked up the bill for short periods in the past, including the ARC which paid 50% of the bill for one year in 1976. "The BRF" said Kirsop "no longer wishes to support the collection because its emphasis has changed over the past ten years. People are using yeasts a lot more and we have constructed a very physiological collection which means we have a great number of yeasts with many interesting and useful properties". The collection holds 10-15 patented strains among a collection of 2000 varieties. "We need to start moving the collection in June, if we are to be out by December," says Kirsop. □

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NEWS IN BRIEF

Europe's biotechnology programme one step nearer

THE European Commission has approved the £16 million, five-year research programme on biotechnology described recently in *Nature* (10 January, page 125). But the programme still has one hurdle to jump before it can be implemented: the Council of Ministers, where the nine EEC member states take final control over Commission proposals.

The Council will take advice from CREST, the Commission's official joint consultative committee for science and technology, in February; and from the European Parliament early in March. If there were no national objections, the programme could be approved in April.

IAEA to hold closed nuclear safety conference

THE International Atomic Energy Agency in Vienna has organised an international conference on current nuclear plant safety issues to be held in Stockholm 20-24 October 1980. Reinhard Schmidt, scientific coordinator of the conference describes it as being within "the framework of those who want and need nuclear power". The conference hopes to address four main issues: assessment of current safety issues, improvements in safety, information exchanges, and development of increased national cooperation. Plenary sessions will be held with "exclusively invited papers" and conference participation "whether or not a paper will be presented must be through designation by the government of a member state of the IAEA". The meeting is expected to be open to the Press although a final decision has not been taken on this yet.

Academy report calls for orderly energy transition

A panel of the US National Academy of Sciences stated last week that both a significant increase in energy efficiency, and the development of new sources of energy — including the fast breeder nuclear reactor — were necessary to make an "orderly and smooth" transition to a coming era of energy scarcity.

The report, prepared by an *ad hoc* Committee on Nuclear and Alternative Energy Systems (CONAES) under a commission from the Department of Energy, emphasised in particular the importance of energy demand considerations in planning future energy supplies.

In a covering letter submitting the report to the department, the two co-chairman, Dr Harvey Brooks of Harvard University and Dr Edward L Ginzton of Varian Associated, emphasise the importance of remembering that the energy problem does not arise from an overall physical shortage of resources.

"The problem is effecting a socially acceptable and smooth transition from gradually depleting resources of oil and natural gas to new technologies whose potential are not now fully developed and whose costs are generally unpredictable", they say.

The report also warns that current uncertainty about the problem associated with the development of nuclear fusion as a source of power means that it cannot be relied upon as a source of energy, at least not until well into the next century.

New head for US Atmospheric Research Center

DR Robert M White, for many years head of the US National Oceanic and Atmospheric Administration and its predecessor organisations, has been appointed president of the University Corporation for Atmospheric Research, responsible for operating the National Center for Atmospheric Research in Boulder, Colorado, under the sponsorship of the National Science Foundation.

Dr White, who will succeed Dr Francis Bretherton as the full-time chief executive of UCAR, is currently administrator of the National Academy of Sciences' National Research Council. He has served as the US permanent representative to the World Meteorological Organisation since 1963, and was chairman of the WMO's world climate conference, held in Geneva in February last year. Dr Bretherton has relinquished his position in order to return full-time to scientific research.

Defector details NATO first nuclear strike plans

THE former senior secretary to the director of war games at NATO's Brussels headquarters said last week that NATO leadership planned to make first use of nuclear weapons in the event of a conflict with Eastern Europe. Ursel Lorenzen, who defected to East Germany last year, told a press conference in Berlin that NATO also had detailed plans to start a war by provoking major incidents such as blocking Eastern European shipping lanes or activating remote controlled mines in international waters. Ms Lorenzen, aged 41, said that for 11 years she had watched the planning and development of strategies to start a war with a rapid nuclear strike against East European nuclear targets. "I was present countless times when high NATO officials and military men confirmed that NATO most certainly in every case would use atomic weapons first" she said. In addition she said that NATO carried out occupation and invasion exercises under simulated Russian winter conditions and that NATO officials had told her that they were drawing on the World War II experience of the Germans. Small NATO member states such as Norway and Denmark who objected to the nuclear strike plans were overruled by the bigger countries, she said.

French police break up researchers protest

A 24 hour occupation of the headquarters of the French national institute of health and medical research (INSERM) by medical researchers was broken up by police at the request of the directors of INSERM last week. Four hundred members of the trade unions representing French medical researchers occupied the headquarters to protest about the government's changes in the status of researchers in INSERM and the Centre Nationale de la Recherche Scientifique (CNRS). The unions called recourse to police action an "illustration of the determination of the institute's director as well as the minister of health and the secretary of state for research not to negotiate". The sensitive points in the new statutes include introduction of a new four year probationary period, a lowering of the maximum age for entry into the profession to 27 years old, and forced mobility at the discretion of research directors.

United Nations University delegation visits USSR

A top level delegation from the UN university visited the USSR from 6 to 11 January at the invitation of Academician D M Gvishiani, vice-Chairman of the State Committee of the USSR for Science and Technology. The purpose of the visit was to establish increased cooperation between the USSR and the university. Talks were described as "productive" but no formal agreements have been finalised. A similar visit to China last May resulted in the creation of two joint research projects in areas of the university's strength — one a study of land-water interaction in the Pearl River basin in China, the other a remote sensing project in the Netherlands — as well as the placing of Chinese scientists for limited periods in western research institutes and universities. "I think we can expect a similar outcome of this visit" a UN official said.

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FEATURES

Middle East brain drain switches back from the West

The brain drain of scientists from the poorer countries of the Middle East has switched from the industrialised West to the oil-rich countries of the region. And some countries are running successful schemes to attract expatriate scientists back.

Ziauddin Sardar reports

THE pattern of the brain drain from the Muslim countries of the Middle East and Pakistan has changed considerably in the past few years. In the sixties and early seventies the flow of scientific and technical expertise was almost exclusively from the Muslim world to the developed countries. As science could not be "consumed" at home or bring adequate financial rewards, the only satisfying career for the up and coming Muslim scientists was in the universities and industries of the West.

This South-North flow of scientific manpower has now given way to a South-South flow: scientists from Muslim countries such as Pakistan are now going to the Middle East. Moreover, many Muslim scientists who had settled in the West or who had trained there are persuaded by the prospect of greater prestige or simply better salaries to return home. Those who have just trained are also finding difficulty in settling in the West because of tighter immigration laws.

The changing pattern of the brain drain is welcomed by scientists and science policy makers in the countries involved. Although no figures are available, the brain drain from Pakistan to the oil-rich states of the Middle East, for example, is thought to be considerably higher than the drain to the West has ever been. But, according to Ghulam Mirza Galani, Secretary of Pakistan's Science Council, "this does not worry Pakistan for two reasons. Firstly, it is not a permanent brain drain. Pakistani scientists working in the Middle East always return home after four or five years, once they have made their money. This contrasts sharply with our brain drain to the West where Pakistani expatriates have tended to settle down. Secondly, while working in the Middle East Pakistani scientists send home much needed foreign exchange so they are not completely cut off from us".

In certain Arab countries the brain drain to oil-rich countries is actually encouraged by the government. Egypt, for example, educates its graduates for the technical-manpower market of oil-rich states. Most Egyptian universities have courses on petrochemicals, desalination and high technology agriculture. Some of these special education programmes are recognised only in the Arab world and a few particular qualifications are only recognised in specific countries. Certain Egyptian medical degrees and doctorates, for example, are valid outside only in Saudi Arabia.

Syria, unlike Egypt, faces an acute shortage of scientific manpower, but it manages to provide facilities for its scientists who wish to work in other Arab states. "Once it used to be the United States, West Germany and France", says Dr Asaad Lutfi, Vice-Rector for Scientific Affairs, University of Damascus, "but now our brightest scientists wish to go to Saudi Arabia, Libya and the Gulf.

"But we do not feel bad about this. It is our responsibility to help other Arab states. We see this as our scientists making an important contribution to the development of the region." Scientists who wish to work in other Arab states are granted up to 4 years' leave of absence; in many cases the government helps them find employment in the country of their choice. According to Dr Lufti over one-third of Syria's scientific manpower is using the scheme.

As exporters of manpower, both Egypt and Syria have an advantage over the other main manpower exporter to the Arab region — Pakistan — being Arabic-speaking. Egyptian scientists seem to favour Saudi Arabia and the Gulf whereas

LEBANESE agronomists testing soil humidity: until four or five years ago, the Lebanon was a major training ground for scientists in the Middle East. The American University in Beirut, in particular, trained many scientists for the region.

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the Syrians prefer Algeria where the language of science is being changed from French to Arabic.

Rich Arab countries are also making efforts to attract the pool of Muslim scientists and technologists working in the West.

Attracting the expatriates home

For example, Libya has set up the Tripoli-based Arab Development Institute — which despite its name is an organisation devoted to scientific research in fields that range from biology to solar energy — and provides it with almost unlimited resources. Although it employs only 70 scientists, it is particularly attractive to Muslim scientists working in the West because of the high salaries — a young PhD can earn $2000 per month — and the excellent facilities. According to Dr Kamel Al Din Hassan, head of solar energy research, "when a project is approved, the scientists simply go ahead and spend what they have to. Research is what counts, budget limitations just do not apply." Publication is also guaranteed in the ADI's own quarterly research journal. The institute now runs recruiting offices in Rabat, London and Washington and is planning opening offices in every Arab capital. The Libyans consider it an outstanding success.

But not all Muslim countries have access to endless financial resources. Turkey, for example, has had to seek alternative ways of attracting back its scientists. In 1977, the Turkish government, with the help of the UN Development Programme, initiated the Project for Transfer of Know-How Through Expatriate Nationals (TOKTEN) with the aim of securing the expertise gained abroad by the many expatriate Turkish scientists and technologists. After two years in operation the TOKTEN project has proved to be ingenious and highly workable.

The main objective of TOKTEN is for specialists of Turkish origin now resident in the West to provide specific technical help. It gives preference to scientists of international standing whose services are obtained for short assignments of one week to a maximum of three months. The visiting scientist transmits his knowledge through training seminars, consultations, reports and proposals on policy issues, and other advisory services. The UNDP meets the travel costs, and living expenses, but pays no salary; the host institution pays an honorarium.

This method of consultancy by expatriates has proved far superior to traditional methods of seeking technical assistance. According to Professor Hakki Oranc, Assistant Secretary General of the Scientific and Technical Research Council of Turkey (TUBITAK), "in general a rich return on the time invested by the consultant has been achieved. There is no need for a period of adaptation, adjustment, acquaintance of the consultant with local conditions, or for the establishment of his credibility. His cultural affinity to Turkey improves his acceptance and effectiveness".

There are further reasons why TOKTEN has proved to be more successful than most multi-lateral technical cooperation programmes. The Turkish expatriates provide a faster response to an institution's problem than is possible through traditional technical cooperation. Sometimes the character and confidentiality of the desired advice requires a privileged relationship which is easier to ensure with a trusted expatriate. Often the full value of a training programme or seminar can be ensured only if it is conducted in Turkish.

Quite a number of expatriate Turkish scientists have become specialists in solar energy research. Some have been brought under the TOKTEN scheme to Turkey for various lengths of time bringing the latest know-how. This should make a significant contribution to the success of a proposed regional centre for the Mediterranean on alternative sources of energy, which the UNDP is expected to support in southern Turkey. The government also hopes to seek the help of an equally large group of expatriate nuclear scientists in its attempt to make Turkey self-sufficient in nuclear power by the turn of the century (see *Nature* **282**, 670; 1979).

"The TOKTEN project has shown that Turkish expatriate scientists are motivated by a desire to help their homeland and not by financial gain", says Professor Oranc. "Of course, we hope that some of them will get so involved with their assignments that they will prefer to work in Turkey. But until such time, scientific and technical gains to Turkey from their efforts are proving to be much higher than one would expect from traditional technical assistance provided at much higher cost", he says.

Second-class citizens

Despite considerable economic gains, Muslim scientists working in the Middle East complain bitterly about the working conditions. The most common complaint is that they are treated as second-class citizens. One Saudi scientist, Dr Raghib El Naggar, an Egyptian says: "expatriate scientists working in Saudi Arabia, the Gulf and Libya are treated viciously. They have no rights whatsoever and virtually no prospects for promotion. No wonder then that most of them leave as soon as they have gained their financial goals". Expatriate scientists in the Middle East are also facing the same problems of corruption and bureaucracy that they are running from in their native countries. "Many expatriates believe that if they have to fight corruption and bureaucracy, they would rather stage the battle back home. There they have a higher probability of success". □

Institut

THE Institut Pasteur is a monument to great men. Pasteur himself is entombed there, in a glorious mosaic sepulchre; images of Faith, Hope, Charity — and Science — gaze down on his casket, and chickens, cows and silkworms decorate the walls. His spirit, and that of the more recently deceased director, Jaques Monod, still inhabit the place.

One of Pasteur's passions was that the new biology — his discovery of the role of microorganisms in disease — should be applied to medicine and agriculture. Biology was not an end in itself but existed to help people, industry, and France. There was a certain practical socialism to his vision, and it remains with the Institut Pasteur today.

Now, François Jacob believes, there is a dual conflict with industry. People in the universities have contempt for applied work, "and industry considers that the scientists are leftist, impossible people".

The Pasteur has a factory that contributes 20% of the funds, with 50% coming from government and 30% from legacies and donations, but negotiations are going on to increase the proportion from manufacture by involving private firms.

Pasteur himself, said Jacob, was able to do all his industrial work in the laboratory, on the bench — "and this was still possible up to the last war; but after the war it didn't work like that at all, and the Institut's funds began to decline".

And despite the Pasteur's history "even here it is not easy to put the people in the factory and the people in the research laboratory together". There is financial encouragement to do applied work, but few Pasteur researchers take it up.

The division is also present in biotechnology. Jacob has recently co-authored a report on the subject with François Gros, Director of the Pasteur, and Pierre Royer, President of the administrative council; they recommend that France's principle research funding body, the DGRST (Directorate Generale pour la Recherche Scientifique et Technique), should control any initiative that France might take.

"We have a secretary of state for science, Pierre Aigrain, and of course we have a minister for industry — and there is already a debate between them over who does what and who gets the money". The DGRST is under Aigrain, and "it is better that they make moves towards industry than industry towards science. But the tendency here is to give the whole thing to industry".

But new firms, such as Biogen and Genentech in the US "are interesting: probably much of this biotechnology is small business anyway, and the small firms may be the best way to put things together . . . In our report we suggested it would be

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Pasteur seeks out industrial support

François Jacob wants industry's money for fundamental biology — but not its control. A Nobel laureate for his work on the *lac* operon (he shared the prize with Jaques Monod), he is head of the cellular genetics unit in the department of molecular biology, Institut Pasteur in Paris. He spoke with **Robert Walgate**

better to push biotechnology with small enterprises, like wine making and so on".

In the laboratory, secrecy and confidentiality were going to be a problem, but the secrecy "will really be extremely limited . . . it will concern what people are actually doing; it has nothing much to do with the basic principles. It may be some trick to get some particular messenger, or something like that; it is unpleasant, because secrecy is always unpleasant, but I don't think it is a severe problem — in this case anyway.

"To know that these guys are doing insulin is not a very big secret; what is important is to know how it works."

"What is worse is that industry is going to control a lot of basic research. Because if, as usual, a researcher has difficulty in getting money, and if he can get money only in what interests industry, industry is going to channel research in its own directions. I think this is more injurious than secrecy."

How could this be dealt with? "Well, the channelled industry money must be kept down to a certain fraction of the research budget. I have nothing against giving *free* money!"

But this would be difficult to police; "and that's exactly what we are fighting with in the Institut Pasteur. We have had troubles with industry since the war; and now there is an attempt to cooperate more narrowly with some big firms.

"We are trying to make arrangements that are not injurious to the Pasteur. The French pharmaceutical industry has done very little for basic research — except for two or three firms. Most of them have done only very narrowly directed, applied research.

"It's always unpleasant to have to negotiate with these people. What they would like to do is to give money to the Pasteur as a function of contracts; but we want the main part of the money to be completely free for basic research."

The problem is the Institut's factory, which is "either too big or too small". The factory has to make vaccines "which are used only in the case of epidemics — to avoid situations like Brazil some years ago when a lot of people died of a meninogococcal infection. The big drug companies are not interested in that. We are making stocks of these things here but there is no profit in them."

"Profit politics will not go on forever — there will be a time when you have to stop this nonsense — but probably the big drug companies cannot do it."

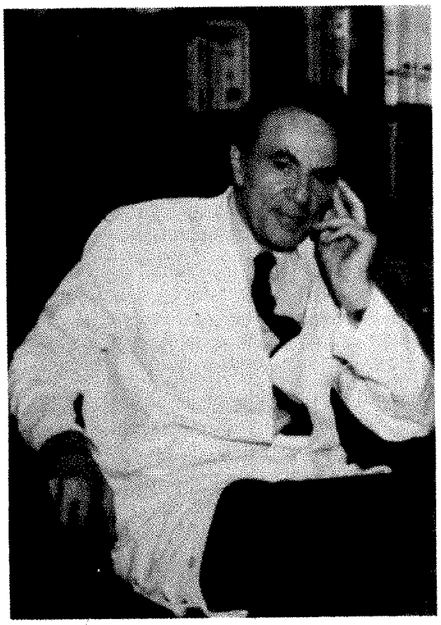
Is there a socialist ethic in the Pasteur? "Yes, that was the idea of Pasteur himself, and Monod tried to pick it up." The mood continues very strongly, "and that's exactly the difficulty of our negotiations with industry".

In the recent report on biotechnology, Jacob wrote the last and most philosophical chapter, on the possible interactions between biology and society in the next 20 years. "The problem was the following. We were asked to produce predictions of the unpredictable . . . and the most interesting thing in science is precisely what is unpredictable.

"What kind of thing could you predict? The first thing I took was the pill, and then the ability to choose a baby's sex, which would not have much impact in Western Europe."

He also predicted brain drugs: two scenarios of psychoactive drugs which would be produced in large quantities through molecular cloning techniques; in one, they would be used by governments as warfare or social control agents; in the other, as a more advanced kind of pill-popping, hyper-valium, opiates of the masses: Aldous Huxley's Brave New World.

"You take this seriously or not seriously as you wish; personally, though, I do not take it seriously."



Francis Jacob — carrying on the traditions of Pasteur.

More serious was the transformation of certain ideologies from biology into political beliefs like social Darwinism or eugenics.

Jacob allows himself one remark however: "the genetic diversity which creates the richness of animal and vegetable species also creates that of the human species," he writes in the report.

"A population composed of individuals genetically too alike would find itself at the mercy of an accident — an epidemic or a sudden shift in the conditions of life. All efforts towards a uniformity in individual biological characteristics, be it towards an 'improvement' of the race through eugenics, or towards the increase of an aptitude for mathematics or for running, would be biologically suicidal and socially absurd".

Scientists should discuss their work, with as little secrecy as possible; they should point out what is possible in five or ten years, even if they think it will not come to pass; and then there have to be enough people who are not scientists making the effort to understand.

"These questions should not be restricted to scientists and/or politicians. Reasonable people from outside must come in — but then they have to make the effort, and the scientists have to make the effort, to talk to each other and understand what is going on."

Professor Jacob proposes that the Academie des Sciences should coordinate such discussions. "I'm not sure they will, but they should". Or there could be a European forum: the European Molecular Biological Organisation could do something, for example.

However, Jacob is not hopeful that France will join in with the European programme on biotechnology presently under discussion in Brussels (*Nature* 10 January p 125).

"The French attitude will probably be nationalistic. But that is just stupid, because I think Western Europe is just going to be eaten in the next 50 years. I'm very pessimistic — aren't you? I just don't see how one can be optimistic. There are many reasons but especially because of this tremendous differential increase in population between Europe and the rest of the world . . . And then there will be the problem of food; you will have some fancy food technology in the West while the rest starve . . . it's a tremendous mess".

François Jacob, who was a Spitfire pilot in the war, and had to get up once or twice during our interview to ease his shrapnel wounds, told me "I personally am very much a European and have always been so since the war; but it's a fact that nationalism is on the increase, not more in England than in France. The whole thing is just incredible". ☐

CORRESPONDENCE

Clerk-Maxwell centenary

Sir, — It is not my intention to spoil the effect of the perfect tribute paid by Professor Domb in *Nature* (15 November, page 235) to Maxwell on the occasion of the centenary of his death. But could I be permitted to make a minor point and to mention Maxwell's ideas about the principle of relativity of motion?

Professor Domb's remark that the true quality of Maxwell's genius became apparent only with the development of twentieth century physics, might lead some to believe that his genius was lost on his immediate successors in the nineteenth century. Far from it. Perhaps, Boltzmann gave the most eloquent praise to Maxwell's equations (of electromagnetism). On reading these equations Boltzmann exclaimed, "War es ein Gott, der diese Zeichen Schrib?" (Was it a God, who wrote these signs?) (*Mensch, Physiker, Philosoph.*) Berlin 1957, page 31).

The word "relativity" was first coined by Samuel Coleridge in the year 1834 but he used it in a philosophical sense. It appears that Maxwell was the first ever to use the word relativity with reference to the doctrine of relativity of motion, and this not only for mechanical but also for electromagnetic phenomena. True, he suggested experiments to determine the putative motion of the Earth relative to the "ether" and in fact made an experiment himself to measure this motion, using a rotating spectroscope, prism and partially silvered mirrors but got a negative result (*Phil. Trans.* 1868, page 532). Therefore Maxwell was not sure whether motion relative to the ether could be observed, but he ultimately moved to a firm belief in the relativity of all phenomena.

It is not widely known that in *A Treatise on Electricity and Magnetism* published in the year 1873, Maxwell devoted two articles, to the modifications of the equations of "electromotive intensity" when referred to moving axes. Using a method which is questionable, he yet came to the conclusion that "the electromotive intensity (in the moving system) is *expressed by a formula of the same type*", and added, "In all phenomena, therefore, relating to closed circuits and the currents in them, *it is indifferent whether the axes to which we refer the system be at rest or in motion*". This was more than an intimation of things to come.

Four years later in the year 1877, Maxwell published the book *Matter and Motion*, in which he used the word 'relativity' and affirmed, "Our whole progress up to this point may be described as a gradual development of the *doctrine of relativity of all physical phenomena.*" Obviously he expected that the principle would be found to be universally valid. Penetrating deep into the future he proclaimed, "There are no landmarks in space . . . we have no log which we can cast to take a dead reckoning by . . . we may compute our motion with respect to our neighbouring bodies, but we do not know how these may be moving in space."

However, he thought as Newton before him did, that the doctrine of relativity broke down in the case of rotational motion, saying, "But though it is impossible to determine the absolute velocity of a body in space, it is possible to determine whether the direction of a line in a material system is constant or variable. For instance, it is possible by observations made on the Earth alone, without reference to the heavenly bodies, to determine whether the Earth is rotating or not". Maxwell then quoted Newton's bucket-experiment, carrying the bucket in thought to the North Pole. However, finding the calculated depression of the water-surface in the bucket too small for measurement, Maxwell remarked that the most satisfactory experimental proof was Foucault's pendulum, the rotation of which (except at the equator) establishes the absolute rotation of the Earth, although the stars and the pendulum are in no visible manner connected.

Probably Poincaré, who developed these ideas soon after, and used the phrase "Le principe de relativité," followed in Maxwell's footsteps — and, of course, travelled farther. Otherwise, how could we explain the exact transliteration of "relativity" into "relativité"?

Yours faithfully,
G. H. Keswani

Tata Institute of Fundamental Research,
Bombay, India

Dioxin detection

Sir, — *Nature* is too important a scientific forum to leave unchallenged the inaccuracies contained in the report (8 March, 1979, page 109) of my comments about comparative laboratory measurements of 2,3,7,8-tetrachlorodibenzo-paradioxin (TCDD or dioxin).

Alastair Hay failed to provide any context to my comments and they appear to be an admission that Dow's analytical capabilities are poor in evaluating the dioxin content of environmental samples. This, of course, is not true. Dow chemists are now confident that the 2,3,7,8-TCDD molecule can be isolated from its isomers and detected at the 5 parts per trillion (10^{12}) level in environmental samples.

With that as background, let me move to the incident reported in your 8 March issue — the EPA study to evaluate the ability of the Agency's Mississippi Test Facility to "spike" and clean up samples of beef fat and human milk; and the determination of abilities of various laboratories (University of Nebraska, Harvard University, EPA's PTSEL Laboratory at Research Triangle Park, Wright State University and Dow Chemical) to measure TCDD in final extracts.

Beef fat was spiked and extracted by EPA Mississippi Test Facility using an acid/base procedure. Identical samples were shipped to Lab A and Lab B. An equivalent set was shipped to Lab C at a different time. Other extracts of beef fat were shipped to Lab D and Lab E. All the laboratories made measurements of TCDD. Of the total 20 unspiked samples measured, 10 gave false positive results. The 15 samples spiked with 0.5 to 9 ppt TCDD showed seven false positive results and zero false negative results. Lab C did not show false results below 9 ppt because its detection limit was too high to make measurements. The 94 samples spiked with 9-81 ppt TCDD showed two false negative results and one false positive.

Spiked beef fat was shipped to Lab D to evaluate its neutral extraction procedure for TCDD. These extracts were examined by Lab D and Lab C. Of six extracts of unspiked samples, one showed false positive results. The 18 spiked below 9 ppt showed six false positive results and zero false negative results. Above 9 ppt, two of 38 extracts gave false positive results and one false negative.

Extracts of human milk were shipped to Lab A and Lab B. Of 12 unspiked extracts, 11 gave false positive results. Of 12 extracts spiked with 0.5 to 9 ppt TCDD, two gave false positive results and three false negative. The 53 extracts spiked above 9 ppt TCDD gave no false positive results and no false negative results.

So the collaborators correctly concluded that the method gave acceptable results above 9 ppt but none of the laboratories was able to produce satisfactory data below 9 ppt.

These facts may be accounted for by any or all of the following reasons: the clean-up may be unsuitable for the particular mass spectrometer being used; the extracts may have stood too long before measurement; or the mass spectrometer may not have been performing properly.

The results obtained in this study in no way compromise results obtained by the various laboratories in other studies in which they did their own extractions and clean up.

Yours faithfully,
Warren B. Crummett

Dow Chemical, Michigan, US

Closed universities better than "sophistry centres"

Sir, — It was obvious that somebody would point out that the 1960s expansion of universities has overstretched resources, and now the words have come, from the mouth of Lord Todd and others. Since the resources derive from the taxpayers, one has only to persuade them that what Lord Todd says is true and it will become so — universities have little else in the way of income, at least as far as scientific research is concerned.

But is the answer to encourage low-caste universities to give up research? Lord Todd is surely as aware as anybody else how research stimulates — even positively goads — teachers into keeping ahead, delving in hidden places and generally searching out the truth. Who benefits most? — the students of course. Without research, no chemistry department is any good at all, and the outcome of Lord Todd's suggestion will be to create not teaching centres but sophistry centres.

If things are so bad we should not be aghast at the idea of closing down a few universities completely; it would be better than trying to keep alive an impotent, wishy-washy, limping sort of place in every university town.

Yours faithfully,
Roy Ballard

Norwich, UK

Iranian terrorists

Sir, — The cynical apology of the Iranian terrorists in *Nature* (29 November, page 439) reminds one of Geoffrey Dawson's infamous editorial in *The Times* of 7 September, 1938. Brutal terror will always appeal to a certain kind of intelligentsia. Let them have their fun, but please spare us the hypocrisy of preaching to the victims and not to the henchmen. Europe no longer has a Churchill to redeem a Munich.

Yours faithfully,
S. V. Vaeck SC

17A rue de la Senne,
1000 Brussels

Islamic science

Sir,—I appreciate that the Islamic world has lived under western colonisation and oppression, particularly economic oppression, for centuries. However, I found that the article 'A revival for Islam, a boost for science?" (22 November, page 354) has a religious bias and is highly racist and chauvinistic. I do not recall any great scientist from Galileo to Einstein mentioning a religious science.

A quotation on page 354 states: ". . . The new civilisation of Islam will be capable of boosting scientific activity and scientific knowledge to heights and achievements unknown to man before". Surely boosting would have been more appropriate round. However, the simplest, scientific question that the statement begs is: why?

Your faithfully,
J. M. Goldschvartz

Rijswijk (ZH), The Netherlands

NEWS AND VIEWS

Dust grain orbits in the Solar System

from David W. Hughes

LONG gone are the days when interplanetary dust grains were thought to move in simple elliptical orbits around the Sun. Today three factors in addition to the gravitational field have to be taken into account in determining their orbits.

First, there is a force acting on the grain simply because it is being illuminated by solar electromagnetic radiation. This force contains a radiation pressure term which is related to the ratio between the amount of solar photon momentum intercepted by the grain and the grain mass. For certain particle sizes and materials this radiation pressure force can even exceed the solar gravitational attraction and the particle can be 'blown' out of the Solar System.

Energy absorbed from the solar radiation flux is reradiated isotropically in the frame of reference of the particle. In the rest frame of the Sun however, more momentum is radiated in the direction of motion of the particle than in other directions and this results in a dynamic drag and deceleration. This is known as the Poynting–Robertson effect. Due to this the eccentricities of the orbits of large particles slowly decrease and the particles spiral in towards the Sun in a time which is proportional to the product of radius and density.

Another, more subtle radiation drag operates due to the rotation of the Sun. Light from the advancing half is blue-shifted, increasing its momentum. Light from the retreating half is red-shifted and the resulting asymmetry in the flux of radiation momentum produces an additional force on the particle transverse to the radiation direction. This force is only significant when the particle is close to the solar surface.

A spinning particle which takes time to attain thermal equilibrium will have a skewed temperature pattern. In other words it will have an evening hemisphere that is on average slightly warmer than the morning hemisphere. The warmer hemisphere radiates more energy and momentum, and the integrated reaction force will have a transverse component somewhat analogous to the non-gravitational forces that perturb cometary orbits. This so-called Yarkovsky effect can increase or decrease the semi-major axis of the particle orbit dependent on the spin direction. The spin speed and particle composition are also important. It is only significant, though, for large particles of over a metre in size.

The second factor affecting the particle orbit is similar to the Poynting–Robertson effect but is caused by the absorption and re-emission of solar wind protons.

The relative significance of the effects mentioned above has been reviewed in a recent paper by J.A. Burns, P.L. Lamy and S. Soter in *Icarus* (**40**, 1; 1979).

The third factor leading to changes in the orbit of a particle is the electromagnetic charge. A force is exerted on a charged particle as it moves in the interplanetary electromagnetic field of the solar wind. This effect has been studied in detail by G.E. Morfill and E. Grün of the Max-Planck-Institüt für Kernphysik, Heidelberg in a recent paper in *Planetary and Space Science* (**27**, 1269; 1979). All dust particles in the heliosphere, a circular region around the Sun extending out to a distance of about 50 AU, have a potential of about 10 V. This constant value is due to the fact that the mechanism causing charging (electron emission induced by solar ultraviolet radiation) and the mechanism responsible for charge decay (electron influx and subsequent recombination) both decrease inversely with the square of the distance from the Sun.

At the Earth's orbit the interplanetary magnetic field B is about 5×10^{-5} gauss. There are strong magnetic fields on the Sun and currents are induced in the solar wind plasma as it moves through them. These currents distort the field and this field is carried away from the Sun just as if it were 'frozen-in' to the moving plasma. The Sun takes 27 days to make one complete turn on its axis and a stream of particles emitted over a period of time from an active area forms a loose spiral in space. The associated magnetic field also has a spiral shape. This magnetic spiral is divided into an even number of sectors and the magnetic field is directed in opposite senses in neighbouring sectors. The solar magnetic field reverses in polarity every 11 years and also the latitudinal extent of the sectors varies in phase with solar activity.

The force acting on the charged particle (the Lorentz force) is proportional to $R\phi$ where R is the particle radius and ϕ is the electrical potential. It is also proportional to $\mathbf{V} \times \mathbf{B}$, the vector product of the relative velocity between the particle and the solar wind magnetic field B, and that field B. Now, as a particle moves from sector to sector in its journey around the Sun the effect of the perturbing Lorentz forces are in opposite directions. However as the sectors are not exactly equal in size and strength, stochastic magnetic scattering occurs. Morfill and Grün consider the effect of this scattering on the orbital inclination of dust grains. It is strongly size dependent. For particles of 10^{-4} cm radii the upper limit is 24° and as the size increases this limit drops, being 3° for 4×10^{-4} cm radii and 0.6° for 10^{-3} cm radii. This result is of importance because it indicates that the thickness of the zodiacal dust cloud, a thickness which corresponds to a root mean square spread of inclination of the order of 20°, cannot be produced entirely by magnetic scattering but must be a characteristic of the source of the dust particles.

Systematic orbital perturbations can be induced electromagnetically due to the fact that the equatorial plane of the Sun and thus of the solar wind magnetic field is at an inclination of about 7.3° to the ecliptic (the plane of the Earth's orbit). The effectiveness of this perturbation depends on the orbital period of the particle. If this is much greater than the solar 11 yr cycle (that is, orbital radii >10 AU) it cancels out. If it is much less (orbital radii <1 AU) then inclination changes occur which tend to pull the orbital plane into the solar magnetic equatorial plane. Particles orbiting inside 0.3 AU may even enter regions of magnetic resonance and this can enhance the orbital changes. However random changes in the sector structure weaken these resonances considerably.

Morfill and Grün make an interesting prediction from their calculations. Particle inclinations are either 'focused' towards

David Hughes is a lecturer in the Department of Physics, University of Sheffield.

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the magnetic equator or 'defocused' during alternate solar cycles. This might be observable as a solar cycle variation in the width or thickness of the zodiacal cloud and should be looked for.

The final problem they consider is that of the inclination of the zodiacal cloud. Recent satellite observations have shown that the cloud is inclined at 3.0±0.3° to the ecliptic, these measurements being taken between 0.3 AU and 1 AU. The two main perturbation forces — gravitation and Lorentz — have symmetry planes inclined at 1.6° and 7.3° to the ecliptic respectively. Jupiter, the main agent responsible for gravitational perturbation, has a very minor effect inside 1 AU. Morfill and Grün conclude that the inclination of the observed plane of symmetry of the zodiacal light is considerably affected by the interaction of the interplanetary magnetic field on the charged dust particles, even if this field distribution doesn't materially alter the thickness of the cloud.

Snags still exist. The particles contributing most effectively to the zodiacal light are thought to have diameters from 10^{-3} to 10^{-2} cm and the magnetic perturbation is strongly size dependent and is small in this range. Also the orbital distribution of the source must have an influence — an influence that decreases as a function of the lifetime of the particle. Certain short-period comets are the main dust source. The 3° tilt of the zodiacal cloud probably results from a combination of the source inclination distribution and the magnetic perturbation but the relative strengths of these influences still need to be calculated. □

The life of a B lymphocyte

by Miranda Robertson

THE ontogeny of the B lymphocyte is unique in that it is marked by a series of changes in gene expression which involve chromosomal rearrangements reflected in a single molecule: the immunoglobulin secreted by a terminally differentiated plasma cell is the end result of a sequence of genetic decisions bearing both on its antigen specificity and its effector action.

The mechanisms underlying this sequence of decisions, and their relationship to other events in the life of a B lymphocyte were among the topics of discussion at a recent meeting* which covered most of the ground between the emergence of the first B lymphocyte in the fetus and the formation of an interacting network of immunoglobulin-secreting cells in the adult.

Commitment to the B cell lineage in avian embryos does not seem to occur until after the blood-borne stem cells have already migrated to the thymus (where T cells differentiate) and the bursa (where B cells differentiate)[1]. The thymus can be shown to release a chemotactic factor which attracts stem cells and is shut off when a certain number have seeded the thymus. Bursal seeding begins only after the thymus is 'full'. But even at that stage, bursal cells are still capable of responding to thymic chemotactic factor: if unseeded thymic tissue is grafted into a chick embryo after bursal seeding has taken place, bursal cells will migrate to the new thymus and differentiate there (N.M. Le Douarin, Nogent-sur-Marne).

The nature of the signals that irrevocably commit a stem cell to a given lineage is not known for either fetus or adult of any species; in mammals, B cell differentiation begins in placenta and fetal liver and continues in adult bone marrow where other haemopoietic cells also differentiate. Commitment to the B cell lineage is first detectable when immunoglobulin heavy chains appear in the 'pre-B' cells of the bone marrow or fetal liver[2]. It is now known that the appearance of heavy chains signifies not only a decision to express immunoglobulin genes, but also a major decision on the antigen-specificity of the cell and its progeny. This follows from the discovery, first made in light chains [3,4] but now extended to heavy chains[5], that the expression of immunoglobulin genes can occur only after a chromosomal rearrangement which determines the primary sequence of the antigen-binding site.

Generation of antibody diversity

J.S. Seidman (National Institutes of Health) reviewed the evidence from mouse κ light chains that there are three modes of encoding antibody diversity. The complete coding region for the antigen binding site is constructed by the recombination of one of between 100 and 1,000 V genes at random with one of 5 J genes, with slight variations in the site of recombination. If similar recombination events take place independently between similar numbers of V and J genes on light and heavy chains, these sources of diversity could in theory account for the entire immunoglobulin repertoire, even without somatic diversification of hypervariable regions 1 and 2; especially in view of the fact that heavy chain expression precedes and is independent of that of light chains, so that a cell committed to a particular heavy chain specificity may give rise to progeny expressing a variety of light chains[2]. Since immunoglobulin is not expressed on the surface of B cells until light chains have been synthesised, this would imply that exposure to antigen plays no part in determining the repertoire, but merely triggers the selective proliferation of cells with an existing repertoire.

Further evidence against later changes in the antigen-binding site can be derived from observations on cultured cells undergoing the ontogenetic changes that follow the initial expression of immunoglobulin. These changes take place in the effector region of the immunoglobulin molecule, coded by seven genes specifying the constant regions of the five classes of immunoglobulin: IgM, IgD, IgG (which has three subclasses), IgA and IgE. All cells initially express IgM, in which the antigen-binding region of the heavy chain is linked to a μ chain. Some may then also express IgD; a smaller number subsequently switch to IgG, and fewer still to IgA and IgE. Antisera against the idiotypes — antigenic determinants on the antigen-binding site — of cultured leukaemic cells, or cells transformed by Epstein-Barr virus, show that they remain unchanged from the pre-B stage through at least one heavy-chain switch, from μ chains to α chains (M.D. Cooper, Alabama University). A possible weakness in this evidence is that it is not clear what determinants anti-idiotypic antisera are recognising. Experiments with monoclonal anti-idiotypes defined with polyclonal sera comprise families of determinants some of which may not be associated with the antigen-binding site (K. Rajewsky, Cologne University; A. Nisonoff, Brandeis University). Thus it is conceivable that examination with batteries of monoclonal antisera would reveal subtle ontogenetic changes not detectable by other means.

Some participants did indeed seriously argue that antigen-binding specificities do change during ontogeny, largely on the basis of experiments by A. Coutinho (Basel Institute for Immunology), who claims that B cell precursors bear idiotypes not associated with immunoglobulin molecules and that antisera against these idiotypes can not only trigger growth and differentiation of the cells but induce the expression of immunoglobulin molecules with different idiotypes. This claim, however aroused considerable controversy.

Signals for antibody production

That there is more than one receptor through which B cells can be induced to grow and differentiate, and that antigen is only one of the signals for these processes is no longer seriously disputed. The receptor through which antigen acts on B cells is immunoglobulin expressed on the cell

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*The INSERM Conference on B Cell Differentiation and Idiotypes took place at Seillac on September 30-October 4 1979.

Miranda Robertson is an Associate Editor of Nature.

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membrane. Other receptors, such as those through which mitogens act, probably bind growth factors released by other lymphocytes. At what stages in ontogeny the cells respond to which stimuli is, however, still unclear, partly because of the problem of isolating cells at a defined stage. F. Melchers (Basel Institute for Immunology) was unable to shed any new light on early stages of B cell ontogeny but believes he has clarified the nature of the cell–cell signals that induce the T-cell-dependent production of antibody in B cells expressing surface immunoglobulin.

Three cell types and at least three signals are needed to induce antigen-specific, T-cell dependent immunoglobulin production by B cells. The three cells are the B cell itself, an antigen-specific T cell, and an antigen-presenting cell. The three signals are, first, the antigen; second, a surface antigen coded by the Ia region of the MHC complex and expressed on all three cells: this antigen must be recognised by the T cell as 'self' for the interactions to work (that is, they are MHC restricted); and third, a growth factor produced as a result of the interaction of antigen, T cell and antigen-presenting cell. Melchers has been able to show that the effect of the growth factor depends on the state of differentiation of the B cell. A small, 'resting' B cell that has not encountered antigen will respond with terminal differentiation to an antibody-secreting cell. After stimulation with antigen, the B cell becomes a larger, activated blast: blast cells respond to growth factor with both proliferation and antibody secretion.

One important implication of this schema is that the growth factor itself need not be antigen-specific: the antigen-specificity of the response resides in the induction of growth-factor production, and the induction of blast cell differentiation in B cells. In view of the controversy which has for some time surrounded the question of whether growth factors are, are not, or are sometimes antigen-specific, a system in which at least the cell types and their responses, if not the signals themselves, are defined, could provide important clarification.

On the other hand, this schema is only one among many and is unlikely to settle the issue definitively. Participants were quick to point out, for example, that at present this result can be obtained only with sheep red blood cells, and not with soluble antigens. The eventual clarification of such interactions may well depend on more and better definitions of subsets of cells.

Heavy chain switch

In the normal course of events *in vivo*, the activation of B cells by antigen probably also triggers the heavy chain switch. There is now mounting evidence that chromosomal rearrangements like that which brings about the V–J join also bring about the heavy-chain switch. Honjo and Kataoka[7] were the first to produce evidence, from the hybridisation kinetics of heavy chain mRNA probes with myeloma DNA, that the switch is accompanied by the deletion of the genes coding for the C regions expressed earlier in ontogeny. If deletion is the mechanism of the heavy chain switch, it follows, first, that the ordering of the C genes on the chromosomes should correspond to the order in which they are expressed in ontogeny; and second, that the switch should be irreversible. Genetic evidence from recombinant mouse strains however suggests that the δ chain gene is closer to the V genes than that of the μ chain (W.E. Paul, National Institutes of Health), which runs counter to the first prediction; and myeloma cells in culture seem to be able to switch back and forth between the three subclasses of γ chain (K. Rajewsky, Köln University), which runs counter to the second prediction.

However, the genetic evidence, while suggestive, is not conclusive, and the spontaneous reversion of the myeloma cells could be due to abnormal or unstable chromosome complements. In the meantime, evidence consistent with deletion continues to accrue from investigations on chromosomal DNA. Restriction analysis shows that J genes are near the μ gene in germline DNA[8], and that μ genes have disappeared from myelomas secreting γ_1 chains, μ and γ_1 genes from myelomas secreting γ_{2b} chains, μ, γ_1 and γ_{2b} genes from myelomas secreting γ_{2a} chains, and μ and all three γ subclass genes from cells secreting α chains[9].

Differences in membrane and secreted Igs

There is now good evidence that the last step in B cell differentiation, too, is marked by a change in the primary structure of the immunoglobulin molecule: membrane and secreted immunoglobulin are not the same. R.M.E. Parkhouse (National Institute for Medical Research, London) has used a charge-shift electrophoretic assay for detergent binding to show that the constant region fragments of membrane immunoglobulin M and immunoglobulin D have hydrophobic regions not present on the secreted molecule; and P. Vassalli (Geneva University) finds differences in the cyanogen bromide fragment patterns of membrane and secreted immunoglobulin M, though he declined to say in which part of the C region the difference lay. This is a matter of some importance because the membrane and secreted molecules seem to be identical at the C terminal[10] and the difference must therefore be internal.

This question is not likely to be resolved by analysis of the chromosomal DNA, which has so far failed to reveal more than one gene for each immunoglobulin class. At present, it is widely suspected that the switch from membrane to secreted immunoglobulin is due to differential processing of the primary RNA transcript in the nucleus. Since each of the four domains of the C region is coded by regions of DNA separated by noncoding intervening sequences, it is easy to see how differential splicing could produce changes within the C region and not only at its C terminal.

If the existence of one gene for each immunoglobulin class is confirmed, then strong support for the theory of differential splicing is provided by evidence for at least two[11] and perhaps as many as four[12] different immunoglobulin mRNAs in the cytoplasm; and B cells, which were among the first cells in which genes were discovered to contain intervening sequences, may prove to be invaluable in clarifying how the intervening sequences are removed in the course of gene expression. □

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100 years ago

AFGHAN ETHNOLOGY

THE events now in progress on the north-western frontier of British India have for the third time in this century directed the serious attention of statemen, historians, and ethnologists to the remarkable people who give their name, or rather one of their names, to the north-eastern division of the Iranian table-land. During the empire of the Sassanides the whole of this region, was known as Khorasan, that is, Khoristan, the Land of the Sun or the East. This term, with the gradual reduction of the Persian sway, has shrunk to the proportions of a province on the north-eastern frontier of the Shah's estates, and has been replaced further east by the ethnical expressions Afghanistan and Balochistan, the lands of the Afghans and Baloches. . . the subjoined rough estimate of the population[of Afghanistan]according to nationalities will show that it is very far from being homogeneous:-

Afghans and Pathâns	Iranian stock	3,520,000
Tajiks	Iranian stock	1,000,000
Hindkis	Hindu stock	500,000
Hazaras and Aimaks	Mongolo-Tatar stock	600,000
Kataghâns	Tûrki stock	200,000
Badakshis	Galcha stock	100,000
Baloches	Iranian stock	100,000
Kizl-Bashes	Tûrki stock	75,000
Kohistanis and Siah Posh	Galcha stock	50,000
		6,145,000

From *Nature* **21**, 22 Jan., 276; 1880.

Exposure to lead in childhood: the persisting effects

from M.R. Moore

THE problem of assessing the long-term effects of environmental lead on the health of both adults and children is bedevilled by the difficulties of measuring exposure over long periods. The only likely contenders for useful measures of long-term lead exposure are concentrations in either bones or teeth and sampling of these presents considerable problems. The more easily measured blood lead concentrations or the various biochemical indicators of lead exposure provide an indication of current or recent levels of exposure only. But in determining the probable damage that will be caused, it is not only the length and severity of exposure that may be important but the age at which exposure occurs. Of all the persons in the community, the newborn child is the most prone to injury from overexposure to lead for several reasons, and the damage that may be caused then will have the greatest long-term social and economic consequences.

There are two distinct problems. First, that of overt lead poisoning, whose sometimes fatal effects are not in dispute. Second, and of greater interest at present, are the insidious effects of long-term lower level lead exposure to which various health effects have been ascribed, such as mental retardation, kidney damage and changes in cardiovascular function, but around which there is certainly some controversy about the levels of exposure at which these changes may begin to take place.

The scientific problem in these circumstances is to try and define some break-point in the continuum of exposure at which one can definitely say that injury has taken place. This is inevitably difficult since lead has always been and always will be present in the biosphere and will be taken into the body in food and drink. As there can be no question of reducing lead exposure levels to zero, scientific and medical policy must be the attainment of minimal risk of exposure.

One of the most important factors determining the eventual body-burden of lead is its rate of absorption from the gastrointestinal tract. Recent studies have clearly shown that this may be considerably influenced by the composition of the diet and is almost certainly greater than the commonly-quoted figure of 10% of the total ingested lead. A similar problem is also found in determining the absorption of inhaled lead. Although initial studies indicated pulmonary absorption leading to a rise in blood lead of 1 μg% for every μg m^{-3} of lead in the air, the most recent studies would suggest that the figure is at least twice this, but is totally dependent on particle size and various other physical constraints, such as the volume of air breathed[1].

The problem of exposure in newborn and young children is particularly important as newly-born children, and indeed other mammals, absorb considerably greater percentages of lead from their diet than they do in adult life[2,3]. And they can be exposed to lead in the environment through several routes. *In utero* the fetus is exposed to lead concentrations which are approximately the same as those found in maternal tissues, as the placenta is a poor barrier to lead[4]. After birth, the situation changes dramatically when two different populations of children can be seen. One is breast fed, the other is fed with prepared milk feeds, usually by reconstitution of dried milk powders with domestic water supplies. The concentration of lead in breast milk is approximately the same as that found in human plasma, that is, about one tenth of the concentration of lead in whole blood, and generally will constitute little hazard to children, even where maternal over-exposure has taken place. The lead concentration of all proprietary dried milk feeds is low. Where however these feeds are made up with water in which there are very variable, but often considerable concentrations of lead, the child may be exposed to very high concentrations of lead at a time of life when his or her developing tissues are most susceptible to any toxic insult. Where concentrations of lead in domestic water supplies are high, which occurs in many areas of the world through soft water supplies being delivered through lead plumbing (see ref. 5 for a recent study) the child is exposed to lead concentrations in the diet which on a weight per kg basis are greater than are likely to be experienced at any other time in his or her life and at a time furthermore, when at least twice as much of this dietary lead will be absorbed compared with later life.

The toxic effects of such exposure are not hard to find. Of all the developing tissues in the body, those most prone to such injury are the brain and the central nervous system which are those least able to repair themselves. A number of studies, both in animals and humans, now provide conclusive evidence that neurophysiological deficit will occur in children over-exposed to lead. The exact degree of over-exposure and change in body lead burden which may result in measurable changes remains to be defined.

The toxic effects of lead on the nervous system in these circumstances are probably biphasic. First, lead affects nervous tissue development and growth[6,7]. These effects are almost certainly irreversible and will result in permanent neurological change, especially where the exposure has taken place early in life. Second, lead acting on various biochemical systems will alter concentrations of neuro-active metabolities. This type of change is probably reversible. Proof already exists that such changes can take place within the haem-biosynthetic pathway with overproduction of δ-aminolaevulinic acid, now shown to be neuro-active[8], and in the adrenergic and cholinergic biosynthetic systems with consequent alterations in neurotransmitter concentrations.[8,9]

In the earliest studies of childhood overexposure to lead it was clear that classroom performance was poorer than in children that had not been exposed. Thereafter, studies showed that mental retardation could be linked with overexposure to lead in drinking water and with high concentrations of blood lead at about the time of birth. Studies in El Paso, Texas linked blood lead concentrations of between 40 and 68 μg per 100 ml with diminished classroom performance (adjusted for age) although there was some controversy over the interpretation of these results.

In the most recent studies a similar degree of diminished learning ability (IQ drop of 4-7 points) and behavioural changes such as diminished attention span, have been associated with increased concentrations of lead in teeth. These studies by Needleman *et al.*[10] and Winnecke[11] are the most convincing evidence to date of low level effects, since the use of tooth lead reflects long-term rather than recent exposure in these children. Children with greater exposure to lead performed significantly less well in the Wechsler Intelligence Scale for Children Test. Although tests for gross motor coordination were not done in Winnecke's study in Germany, changes in classroom behaviour were observed in Boston by Needleman *et al.* The importance of these studies lies in the battery of tests applied and in the matching and allowance for non-lead related variables such as weight, height and socioeconomic class, which were controlled in the analysis of covariance.

The emphasis now being placed on the study of the effective dose–response relationship between lead exposure and possible neuropathological effects in children reflects the fact that the overexposure to lead of young children will have the greatest potential economic

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M. Moore is in the Department of Medicine, Western Infirmary, Glasgow.

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effects through increased requirement for care in later life in those most severely afected and by diminution of intellectual potential. Children are inevitably the unwitting victims of adults' use of lead, especially in industrialised communities and in areas of heavy traffic.

It is impossible to put any value whatsoever on human potential and development but it is safe to say that for any community, the economic burden of looking after persons with mental deficit is high and is increasing. This burden should certainly not be unnecessarily loaded with persons in whom such deficit is avoidable.

In industry, the hazards of over exposure to lead are easily perceived and a solution simple, even if expensive to achieve. But this does not apply to environmental exposure. The simplest cause of environmental exposure to remove would be lead in automobile fuel. This course of action would certainly carry a high potential economic toll and lead in petrol is only one limited source of exposure in Western society. To remove other sources such as lead plumbing would be even more expensive, which must inevitably be balanced against the less easily quantified cost of doing nothing. Perhaps the ultimate aim of any economic as opposed to medical policy in this sphere must be to balance the cost of a healthy community against the costs of achieving a healthy environment. It is particularly poignant as the International Year of the Child comes to an end that children who have so little economic or political muscle, but in whom the toxic effects of over exposure to lead are most manifest should thus have the greatest potential long-term economic effect on the community. □

Body temperature, activity and energy costs

from R.D. Martin

LIVING birds and mammals are distinguished as a group by their maintainance of a high body temperature with the generation of heat in association with a high metabolic turnover. Until recently, however, attempts to provide a convincing hypothesis to account for the evolution of this phenomenon of endothermy have remained inconclusive. Now, information from a number of disparate fields of research seems at last to point the way towards a synthetic theory accounting for the emergence of endothermy despite its high energetic cost.

A major stimulus for re-examination of possible reasons for the evolution of endothermy in birds and mammals comes, strangely enough, from a recent suggestion that this feature had also been developed among the dinosaurs (Bakker *Evolution* **25**, 636; 1971; *Nature* **238**, 81; 1972). Bakker's arguments supporting endothermy in dinosaurs (invoking raised body posture, inferred predator/prey ratios indicating high energy requirements, and bone histology indicative of rapid growth) have encountered criticism from several quarters, but regardless of its specific merits the hypothesis had the advantage that it focused attention more on the topics of locomotor activity, energy requirements and overall metabolic turnover. As Hopson (*Paleobiology* **2**, 271; 1976; *Rev. ecol. Syst.* **8**, 429; 1977) concluded, it seems likely that the dinosaurs were not comparable with birds and mammals in terms of body temperature regulation, but they were undoubtedly peculiar in some aspects of their metabolic house-keeping in comparison with modern reptiles. Thus, any synthetic explanation of the origins of endothermy should ideally account not only for the relevant features of birds and mammals, but also for any identifiable peculiarities of the dinosaurs concerning overall energy relationships.

In diametric opposition to the synthetic trend stimulated by Bakker's work, a special explanation for the evolution of endothermy in mammals alone was subsequently proposed by A.W. Crompton, C.R. Taylor and J.A. Jagger (*Nature* **272**, 333; 1978). Their model involved a two-step acquisition of endothermy: (1) invasion of a nocturnal niche, with development of a moderately elevated, controlled body temperature (25-30 °C), but without any significant increase in resting metabolic rate; (2) re-invasion of a diurnal niche, with further elevation of the controlled body temperature (to close to 40 °C) and an obligatory increase in resting metabolic rate.

One important piece of evidence cited in support of this hypothesis consisted of new experimental evidence that three extant placental insectivores (*Tenrec ecaudatus; Setifer setosus; Erinaceus europaeus*) exhibit a 'reptilian-type' pattern of oxygen consumption during locomotion. This feature was intepreted as a primitive retention in these nocturnal insectivores illustrating the first step in the acquisition of endothermy. However, a nocturnal monotreme (*Tachyglossus aculeatus*) and a nocturnal marsupial (*Didelphis virginiana*) studied under the same conditions exhibited a typical 'mammalian-type' pattern of oxygen consumption during locomotion. Thus the two-step model can only account for the conditions in these two species (and for the vast majority of nocturnal placental mammals which presumably have 'mammalian-type' energetics) by postulating numerous intermediate diurnal ancestors in which the second step would have been accomplished. An alternative explanation is that the tenrecs and the hedgehog exhibit 'reptilian-type' energetics during locomotion for special reasons unrelated to the overall framework of evolution of endothermy in mammals. In any event, the two-step model cannot be applied to the parallel evolution of endothermy in birds (for which no ancestral nocturnal phase can be envisaged) and Crompton *et al.* themselves point out that their hypothesis explicitly rules out any possibility of early trends towards endothermy among therapsids (mammal-like reptiles).

The need for an *an hoc* explanation for the separate evolution of endothermy in mammals has now been questioned in a paper by A.F. Bennett and J.A. Ruben (*Science* **206**, 649; 1979), which concentrates on the high energetic cost of endothermy and the relationship between high metabolic rate and increased capacity for aerobic respiration. The high energetic cost of endothermy in birds and mammals demands that it have a substantial selective advantage. Bennett and Ruben propose that this advantage lies in the far greater stamina in locomotion permitted in vertebrates with high metabolic rates and an associated greater capacity for aerobic respiration.

Taking the same linear relationship between oxygen consumption and speed of locomotion exploited by Crompton *et al.* in their experimental work, Bennett and Ruben have shown that a plateau of oxygen consumption is reached at a critical speed of locomotion. For any given body weight, a reptile will attain this plateau at a much lower speed than a mammal and thus be constrained to carry out anaerobic respiration at an earlier stage. (Interestingly, the relationships between oxygen consumption and running speed determined for the placental insectivores studied by Crompton *et al.* reveal no attainment of an anaerobic plateau, so these mammals are apparently not of the 'reptilian-type' in this respect). Hence acquisition of greater locomotor stamina may well provide a unitary explanation for the evolution of endothermy in the face of greatly increased energy consumption.

Two important corollaries are identified by Bennett and Ruben. First, since locomotor stamina is undoubtedly a prerequisite of bird flight, it would follow that endothermy probably evolved among the ancestors of birds before the emergence of flight. Second, since increased metabolic turnover is directly related to a decrease in the proportion of resources which can be allocated to reproduction by any individual, it is likely that endotherms would be particularly subject to selection pressure favouring the emergence of

R.D. Martin is Reader in Physical Anthropology in the Department of Anthropology, University College, London.

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secondary mechanisms to enhance infant survival. The characteristic presence of parental behaviour in birds and mammals (associated with vivipary in the latter) may thus be linked to the presence of endothermy.

Going beyond the material presented by Bennett and Ruben, it is also noteworthy that there is probably a close connection between metabolic turnover, activity levels, aerobic capacity and brain size in birds and mammals. The fact that birds and mammals have relatively larger brains than reptiles, amphibians and fish (Jerison *Evolution of the Brain and Intelligence,* 1973) could relate directly to greater locomotor capacity and to the high metabolic levels associated with endothermy. If a single such explanation covering all these aspects is applicable to living birds and mammals, these corollaries (and others as yet unidentified) could considerably increase our understanding of vertebrate evolution. The present controversy over the presence or absence of endothermy in dinosaurs, having stimulated the search for new explanatory principles, may itself find an acceptable solution within this unitary framework, bringing in such topics as relative brain size (Hopson *op.cit.,* 1977; Jerison *op.cit.,* 1973) and evidence for parental behaviour in dinosaurs (Hopson *op.cit.* 1973; Horner & Makela *Nature* **282,** 296; 1979). ☐

Switchboards and folds

from P.D. Calvert

SEMICRYSTALLINE polymers derive their useful properties of strength and toughness from their structure of alternating crystalline and rubbery layers. Hence the way in which the chains are arranged in these layers and bond them together is of importance in guiding our thinking about many aspects of their behaviour.

Small angle neutron scattering (SANS) results on crystalline polyethylene published in 1976 showed that current ideas on the arrangement of the molecular chains in crystalline polymers needed rethinking (see *News and Views* **263,** 371; 1976). Polymer crystals are usually thin, about 10 nm, in the direction traversed by the chains so it is agreed that chains must fold back for several traverses through any crystal. Before 1976 it was widely believed that the chains were folded tightly to give adjacent re-entry with little loose material at the crystal surface. The alternative was the 'telephone switchboard' model where the chains re-entered the crystal at some distance from their exit point. The Faraday Discussion on 'Organisation of Macromolecules in the Condensed Phase' held in Cambridge last autumn gave a clear picture of developments over the past 3 years.

The basic observation by neutron scattering, that there is little change in the radius of gyration of polyethylene on crystallisation, was originally reported by Ballard and coworkers at ICI. They have since shown that polypropylene behaves similarly and workers at Bristol and at Mainz have confirmed the polyethylene result. At Cambridge, J. Guenet, C. Picot and H. Benoit from Strasbourg reported similar results for deuterated polystyrene chains in a high molecular weight ($M_w = 1.7 \times 10^6$) polystyrene matrix. However, in a matrix of lower molecular weight ($M_w = 4 \times 10^5$) the radius of gyration increased by up to 40% on crystallisation. This is of great interest because the fact that no change in radius of gyration is usually observed means that there is little with which really to test theories. One problem with SANS is that segregation of the labelled molecules may occur on crystallisation, but the Strasbourg group claims that this is not so in their samples.

Several different approaches have been successful in fitting the SANS data. D. Yoon and P. Flory (Stanford University) have shown that switchboard models with or without some low probability of adjacent re-entry can fit the data. The Mainz group has used a 'solidification' model in which straightening of chain sections is superimposed on the initial, molten random coil. The Strasbourg group has fitted its data by allowing adjacent re-entry over the central sections of the chain and leaving amorphous 'wings' at either end. The US National Bureau of Standards group here had success with this and with a cluster model, where clusters of three or four adjacent folds are linked by longer loose folds. The lesson seems to be that the many different models can be fitted to the data available so far, which can only be used to eliminate some possibilities. However, matters should improve as measurements are extended up to 'intermediate' angles where the scattering vector is commensurate with the individual folds and clusters rather than the whole chain. Careful measurements of absolute, rather than relative, intensity may also be necessary in these regions.

The ICI/Jülich group has looked carefully at the behaviour of the radius of gyration at low molecular weights in polypropylene (*Polymer* **20,** 399; 1979). In principle, the chain might be expected to resemble a straight rod when it becomes so short that it can only fold once or twice. Then the rod length should be equal to the lamellar spacing seen by small angle X-ray scattering. In fact it is about twice this. This they have explained in terms of chains running through pairs of lamellae. However, we are rather ignorant about the way in which the stacks of lamellar crystals form; it often seems to be a two-step process with later filling-in by small lamellae of the spaces between those which form first. Thus it is difficult to see how really to view this interesting result. This group also reported data on drawn polypropylene where a six-fold draw ratio resulted in a decrease of 3–4 times in the radius of gyration perpendicular to the draw direction, more than the decrease of $\sqrt{6}$ that might be expected. There was also a decrease of 3–6 times in the molecular weight measured by SANS although the chains were unbroken suggesting that the chain was now acting as a series of separate beads.

Given that interpretation of the SANS data on melt-grown crystals is very dependent on the models used, one would hope for signs of theoretical advances. Here the meeting at Cambridge had little to offer. The interpretation of the results of other experimental observations in terms of the switchboard and adjacent re-entry models were debated, as they have been for many years, but little progress has been made. A great deal of information exists but most of it can be interpreted in terms of more than one model. However, it still remains unclear whether the telephone switchboard model can be reconciled with sensible packing densities close to the crystal surface. Recently Flory suggested that there was not time for a full adjacent re-entry conformation to be adopted during crystallisation, given the relaxation time of a whole chain. J. Klein and E. Di Marzio argued against this, the former by calculating the diffusion rate of a chain in a tube and the latter by calculating the time to pull a chain through a hole in a plane, but as all the calculations are on different bases it is difficult to grasp the reasons for the contradictions. A telling point is that polymeric impurities can be pushed aside even during rapid crystallisation. It is worth giving more thought to the relationship between the time required for this process and that for crystallisation.

Studies on solution-grown polyethylene single crystals seemed to have reached a reasonable level of agreement. Yoon, D. Sadler (Bristol) and M. Stamm (Mainz) all talked in terms of chains re-entering close to their point of exit and spread over a few neighbouring crystal planes, in other words not 'adjacent' re-entry but 'nearby' re-entry. However, there are still differences in the form of the models used by the different groups.

The SANS results have changed most people's picture of the crystal surface. They now picture it with many loose loops whereas previously the majority viewpoint was that the surface was virtually smooth. For the future we must await more detail from the neutron scattering at intermediate angles. The most viable theoretical approach seems to me to be to start from the molten random coil and to interpret the action of the kinetics and thermodynamics of crystallisation and annealing but progress has been slow so far. ☐

Paul Calvert is a lecturer in the School of Molecular Sciences, University of Sussex.

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REVIEW ARTICLE

Thermal X-ray emission from neutron stars

David J. Helfand, Gary A. Chanan & R. Novick

Columbia Astrophysics Laboratory, Departments of Astronomy and Physics, Columbia University, 538 West 120th Street, New York, New York 10027

The Einstein Observatory (HEAO 2) presents the first realistic opportunity to see a neutron star by observing radiation from its surface. In this review we show that the detection of such thermal radiation or the establishment of strong upper limits to neutron star temperatures is of considerable importance to the study of neutron star matter, neutron star structure, and polar magnetospheres.

WHEN the existence of neutron stars was first suggested[1-3], it seemed likely that these objects, with a radius of only 10 km, would forever remain in the realm of theoretical speculation. Observational advances in the past decade have rendered this view obsolete. Neutron stars have been either established or suggested as the underlying object for many astrophysical phenomena: pulsars; X-ray binaries, transients, and bursters; γ-ray bursters; and even active galactic nuclei. Nevertheless, with the possible exception of the decaying tails of X-ray bursts[4], we have yet to 'see' a neutron star in the sense of detecting its surface radiation[5]. Such an observation may well now be possible.

We review here the status of theories concerning the thermal properties of neutron stars with a view towards establishing how observational astronomy may contribute to future studies. Table 1 presents the photon fluxes expected in several regions of the spectrum from a 10 km neutron star at a distance of 100 pc (equal to that of the closest radio pulsar). The legend to Table 1 lists the limiting sensitivities of the best available instruments in each spectral regime. While the Space Telescope will substantially lower sensitivity thresholds in the visible and near UV, much work can already be done in the soft X-ray region with the imaging telescope aboard the Einstein Observatory (HEAO 2).

There are several reasons why neutron star surfaces are thought to be hot enough to emit soft X rays ($T \sim 10^6$ K). At its formation in the imploding core of a supernova, a neutron star will be at a temperature $>10^{11}$ K. It cools rapidly at first through various neutrino processes, but most recent calculations[6] suggest that the surface temperature remains above one million degrees for at least 10^3 yr and above 10^5 K for perhaps 10^5 yr. If this is the case, several neutron stars should be detectable in young and/or nearby supernova remnants. Furthermore, several heating mechanisms have been proposed which may maintain surface temperatures of several hundred thousand degrees or higher in older neutron stars (such as radio pulsars). These include heating of the polar caps via bombardment of the surface with relativistic particles from the magnetosphere, frictional dissipation from the crust–superfluid interface, microquakes and accretion from the interstellar medium.

Heating sources

Initial cooling: Although neutron stars are thought to form at very high temperatures ($T > 10^{11}$ K) in the core of a supernova explosion, much of this initial thermal energy is radiated away in neutrinos during the first hours, leaving a 1-day-old star with an internal temperature of 10^9–10^{10} K. The thermal history of neutron stars has recently been reviewed by Tsuruta[6]. Cooling rates were calculated for various neutron star equations of state, stellar masses and magnetic field strengths. Both photon and neutrino cooling were considered; neutrino processes included were neutrino bremsstrahlung, modified URCA and plasmon processes, as well as minor processes such as photo production of neutrinos, pair annihilation and neutrino synchrotron radiation. The effects of the star's magnetic field and the superfluid nature of its interior were taken into account. In addition to a range of standard equations of state, stars containing pion condensate cores were considered. The thermal properties of more exotic equations of state, including those for pure quark stars, have been studied by Brecher and Burrows[7].

The result of such cooling calculations is a curve giving the neutron star surface temperature as a function of time. Neutrino cooling dominates for at least the first 10^3 yr in all models, so any effect of the magnetic field on photon opacity is of minor consequence. The range of temperatures allowed by standard nuclear models after 350 yr is from 3.5 to 15×10^6 K; after 10^4 yr, the temperatures fall to between 2 and 5×10^6 K. As the result of a less sensitive temperature dependence (T^6 instead of T^8 as for other major processes), neutrino cooling due to pion decays allows the star to reach lower temperatures more rapidly. Although the density ρ_π at which neutron star matter makes a transition to a pion condensate is very uncertain (3×10^{14} g cm$^{-3} \lesssim \rho_\pi \lesssim 3 \times 10^{15}$ g cm^{-3} with $\rho_\pi \sim 6 \times 10^{14}$ g cm^{-3} as the best current estimate[8]), one can compute the maximum cooling effect of pions by choosing the lower limit of 3×10^{14} g cm^{-3}, thus allowing the largest fraction of the stellar mass to be in this state. These maximum pion cooling models yield temperatures of 8×10^5 K and 3×10^5 K after 300 yr and 10^4 yr, respectively[6].

An analytical estimate of the cooling equations by Brown[9] yielded a surface temperature for the Crab pulsar of $\sim 4 \times 10^6$ K, consistent with the results of Tsuruta's[6] full numerical integrations and only slightly above the measured upper limit of 3.0×10^6 K (ref. 10). Malone[11] and Maxwell[12,13] have also studied the cooling problem, and the results are all qualitatively similar. They differ by less than a factor of 2 from the results described above—the discrepancies apparently arise from small differences in the input physics: for example, the choice of an equation of state (Maxwell[12] assumes a uniform density star). However, many of these calculations use core–crust thermal conductivities calculated over seven years ago[14]. Recent work by Ray[15] finds significantly lower conductivity values and thus predicts lower temperatures for recently formed stars. His most extreme model predicts surface temperatures of 1.7×10^6 K and 1.3×10^6 K for stars of age 300 yr and 1,000 yr, respectively. Also, since the core-to-surface thermal conduction time scale is $\sim 10^3$ yr, one cannot distinguish, in these models, between young stars with and without pion condensate cores.

Brecher and Burrows[7] have recently made the first detailed calculations for the cooling of quark stars. These stars tend to have a higher heat capacity, but also tend to cool slightly faster, resulting in predicted temperatures quite similar to those discussed above; a 10^3-yr-old star has a temperature of $\sim 5 \times 10^6$ K. As a result of their higher heat capacity, the stars also tend to remain warm longer. For instance, after 2 Myr (a typical age for radio pulsars), the surface is still at 10^6 K. However, it is uncertain whether a quark star contains a large superfluid component, and, as for normal matter stars, the inclusion of superfluidity effects in these calculations would probably lower these predicted temperatures. The thermal properties of the recently proposed 'abnormal' matter neutron stars[16] have not yet been calculated.

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Table 1 Thermal neutron star photon fluxes in the visible, far UV, and X-ray bands*

T (10^6 K)	Flux Visible band†,‡ (3,000–10,000 Å) (mag)	 Far UV band‡ (1,000–2,000 Å) (mag)	 X-ray band§ (0.2–3.0 keV) (photons $\mathrm{cm^{-2}\,s^{-1}}$)
0.1	28.5	25.8	1.3×10^{-11}
0.3	27.3	24.1	1.9×10^{-4}
1.0	26.0	22.7	0.23
3.0	24.8	21.4	12

* A neutron star radius of 10 km and a distance of 100 pc are assumed; interstellar absorption has been neglected.

† Skylight limits the sensitivity of ground-based telescopes to $m_v \sim 23$.

‡ The wide field camera on the Space Telescope is expected to be able to detect 27th mag stars in the far UV and visible regions of the spectrum.

§ The imaging proportional counter on the Einstein Observatory has a sensitivity of 2.5×10^{-5} photons $\mathrm{cm^{-2}\,s^{-1}}$.

Virtually all of the standard calculations of neutron star cooling rates, therefore, suggest surface temperatures of several million degrees for a 300-yr old object, with temperatures remaining within the range $5\text{–}20\times10^5$ K for at least 10^4 yr. The only previous observation applicable to these theories has been the upper limit of $\sim3\times10^6$ K for the Crab pulsar from the lunar occultation measurements[10,17]. With the imaging capabilities and thousandfold increase in sensitivity offered by the Einstein Observatory, we can now expand this data base significantly. A search of nearby supernova remnants will either detect point sources and yield surface temperatures to be used as constraints on cooling theories, or provide upper limits nearly an order of magnitude lower in temperature than previously possible and delimit the viable choices for neutron star equations of state (for example, by distinguishing between the presence or absence of pion condensates).

Figure 1 presents the minimum surface temperatures required for neutron stars to be detected in 10^4-s pointings with the Einstein Observatory imaging proportional counter (IPC) (see ref. 18 for a detailed description). These temperatures were determined by requiring that the number N of photons in a 2 arc min radius detection cell be at least 25 [a 3 σ result for the typical measured IPC background rate of 0.005 counts (s cell)$^{-1}$]. For a thermal isotropically radiating neutron star at a distance d with temperature T_0 and radius R_0, N is given by

$$N(T_0)=\frac{2\pi(1+z)^2R_0^2t}{c^2d^2h^3}\int_{E_\infty^{(1)}}^{E_\infty^{(2)}}\times\frac{E_\infty^2A(E_\infty)\tau(n,E_\infty)}{\exp[(1+z)E_\infty/kT_0]-1}\,\mathrm{d}E_\infty \tag{1}$$

where t is the observing time. The integral is over photon energies E_∞ within the bandwidth, $E_\infty^{(1)}$ to $E_\infty^{(2)}$, of the detector. The subscript ∞ emphasises that the photon energies are as measured by an observer at infinity. On the other hand, T_0 and R_0 (the 'true' temperature and radius) are defined, in the usual convention, at the surface of the neutron star. We have used the relationships

$$\begin{aligned}T_\infty&=(1+z)^{-1}T_0\\R_\infty&=(1+z)R_0\end{aligned} \tag{2}$$

where T_∞ is the temperature measured by the distant observer and R_∞ is the apparent radius that he would deduce from the black body law (see ref. 19). The factor $(1+z)$ is due to the gravitational redshift and, at the surface of the star, is given by

$$1+z=\left(1-\frac{2GM}{c^2R_0}\right)^{-1/2} \tag{3}$$

where M is the mass of the star (see ref. 20). Finally, $A(E_\infty)$ is the effective area of the detector and $\tau(n, E_\infty)$ is the transmission of the interstellar medium as a function of the number density n of neutral hydrogen atoms along the line of sight. We have plotted results for $n=0.3$, $n=1$, and $n=3$ atoms $\mathrm{cm^{-3}}$. The absorption coefficients used were those of Brown and Gould[21].

For all of the present calculations, we have adopted a neutron star mass of $M=1.3M_\odot$ and a radius $R_0=16$ km. This value for M is used in much of the theoretical work described above and is also consistent with the measured masses of neutron stars found in binary systems[22,23]. The adopted radius is that appropriate to this stellar mass, using either the mean field[24] or tensor interaction[25] equations of state[26]. The value of the redshift for this case is $z=0.13$.

Our assumption of isotropic black body emission for the thermal radiation from a hot neutron star is clearly an oversimplification. The strong magnetic field produces a nonuniform thermal conductivity over the surface of the star leading to significant temperature gradients. Brinkmann[27] has also recently described the deviation from a black body spectrum which results from the effect of the field on the optical properties of the surface. He finds that the effects are not large for stars with fields $\gtrsim10^{12}$ G, but the strong dependence of the surface index of refraction on the values of the cyclotron and plasma frequencies causes the expected radiation to be lowered by more than a factor of 5 from the black body value for $B\sim5\times10^{11}$ G. A measurement of the actual emitted spectrum would reveal much about the properties of neutron star surface material and the strength of the magnetic field.

Polar cap heating: The origin of pulsar radio emission has been one of the most difficult theoretical problems in astrophysics of the past decade. However, models have emerged which agree on several fundamental points. Based on the original work by Sturrock[28], these models invoke pair creation by the γ rays produced via the electrostatic acceleration of primary particles from the magnetic polar caps of the star. Although the location of the region of pair production and many other details of the models differ[28–35], all of the models require that a large back-flux of γ rays and relativistic particles be incident on the pulsar's polar cap surface. This flux will heat the polar cap to soft X-ray temperatures (0.1–1 keV) and, assuming that a large fraction of this energy is not lost by heat conduction into the stellar interior, may render the polar cap region visible at distances of up to ~1 kpc. Owing to uncertainties in the distance from the stellar surface at which pulsar radiation in various spectral regimes arises and the values of some parameters in the polar cap theories, we ignore general relativistic corrections in this section; that is, we drop the distinction between R_0 and R_∞, T_0 and T_∞, and so on.

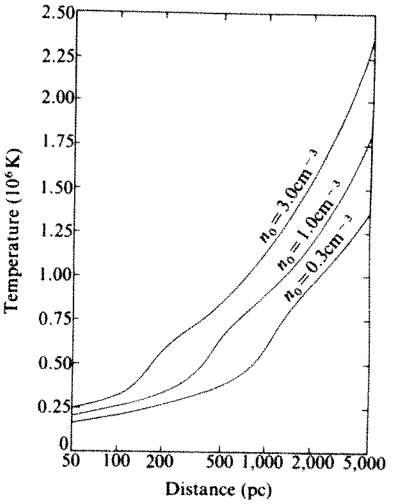

Fig. 1 Temperatures for a 16-km neutron star that produce detectable soft X-ray fluxes (25 counts) in a 10^4-s pointing of the Einstein Observatory IPC. Curves for several representative values of the interstellar neutral hydrogen density, n_0, have been plotted for T as a function of distance. Note that there are 50 known radio pulsars within 500 pc of the Earth.

Table 2 Predicted X-ray temperatures and fluxes for potential γ-ray emitting pulsars

Name PSR	B_{12}/P^2	D (pc)	$\dot{E}$ (10^{33} erg s^{-1})	A (10^{10} cm^2)	T (10^6 K)	IPC counts in 2,000 s (0.1–5 keV)
0355 + 54	35	1,500	46	1.7	7.0	900
0531 + 21	3,400	2,000	4.6×10^5	8.2	7.6*	2,900*
0540 + 23	33	2,800	41	1.1	7.6	180
0611 + 22	40	3,500	64	0.82	9.1	140
0740 − 28	61	2,000	150	1.6	7.6*	570*
0833 − 45	430	500	7,000	3.0	6.7*	16,000*
1055 − 52	31	1,100	37	1.4	7.0	1,500
1449 − 64	50	2,600	97	1.5	8.7	450
1557 − 50	27	9,000	28	1.4	6.5	5.8
1641 − 45	15	4,900	8.5	0.59	6.0	9.6
1727 − 47	17	5,900	11	0.32	7.4	7.2
1742 − 30	15	2,900	8.6	0.73	5.7	38
1826 − 17	14	9,400	7.7	0.87	5.3	1.3
1831 − 03	11	9,400	5.1	0.39	5.8	0.87
1844 − 04	16	4,900	9.7	0.45	6.6	11
1907 + 10	11	5,000	4.6	0.96	4.5	4.1
1913 + 10	15	8,400	9.2	0.67	5.9	2.3
1914 + 09	11	2,000	5.1	1.0	4.6	53
1914 + 13	13	7,900	6.5	0.96	4.9	1.6
1915 + 13	32	3,200	39	1.4	7.0	120
1916 + 14	11	1,000	5.1	0.22	6.7	250
1929 + 10	10	110	4	1.2	4.2	29,000
1930 + 20	15	6,900	8.7	1.0	5.3	3.7
1930 + 22	140	8,100	770	1.9	14	130
1933 + 16	12	6,000	5.2	0.75	5.0	3.1

* Values calculated using measured values of L_γ (see text). In other cases, $L_\gamma = \frac{1}{2}\dot{E}$ was assumed.

The models of Ruderman and coworkers[29-32] offer a choice of two regions of the magnetosphere from which polar cap-heating particles emerge. In the so-called 'outer gap' picture[31,32], a large flux of positrons (electrons) originates in a region of magnetospheric charge reversal at a radius of $\sim 0.2\ R_c$ (where R_c is the light cylinder radius) and impinges on the polar cap, heating it to temperatures of $\sim 10^7$ K. This model accounts well for the high energy γ radiation from the Crab and Vela pulsars as well as for the 100 MeV γ-ray pulses recently reported from two older pulsars (PSR0740 − 28 and PSR1822 − 09)[36]. The condition for such outer-gap induced γ-ray emission is

$$B_{12}/P^2 > 10 \tag{4}$$

where B_{12} is the pulsar's dipole magnetic field strength in 10^{12} G, and P is the period in seconds[32]. Of the 110 pulsars in the Galaxy with measured period derivatives (necessary to estimate B_{12}), the 25 sources which satisfy this criterion are listed in Table 2, along with their values of B_{12}/P^2, distance, and spin-down energy loss rates. From the four observed γ-ray pulsars, it seems that the efficiency with which the rotational kinetic energy lost by the star is converted to γ rays, $\eta = L_\gamma/\dot{E}$, is a strong function of B_{12}/P^2: $\eta = 1/3{,}000$ for the Crab ($B_{12}/P^2 = 3{,}500$), $\eta = 1/200$ for Vela ($B_{12}/P^2 = 425$), $\eta = 0.2$ for PSR0740 − 28 ($B_{12}/P^2 = 60$, and $\eta = 1.0$ for PSR1822 − 09 ($B_{12}/P^2 = 11$). For nearly all of the pulsars listed in Table 2 which have not been detected as γ-ray emitters, B_{12}/P^2 is in the range 10–50. By adopting a value of $\eta = 0.5$ for these sources, we can calculate the expected γ-ray flux at the Earth for each object: in every case, the expected flux is below the threshold detection level for COS B of $\sim 5\times10^{-11}$ erg cm^{-2} s^{-1}, so the supposition that all of these sources may display outer-gap discharges is not in conflict with present γ-ray observations.

M. A. Ruderman (personal communication) estimates that the thermal X-ray heating L_x of the polar cap resulting from the bombardment of charged particles will be $\sim 0.1\ L_\gamma$. This assumption, together with L_γ and the polar cap area, defines the temperature of the polar cap. For the area A of a single polar cap shown in column 5 of Table 2, we have used[37]

$$A = \pi R^2(\Omega R/c) \tag{5}$$

where Ω is the pulsar angular spin frequency, c is the velocity of light, and we take $R = 16$ km as before. Column 6 shows the derived temperature of the polar cap [see equation (7)], where we have used the measured value of L_γ for the four γ-ray pulsars and have taken $L_\gamma = \frac{1}{2}\dot{E}$ otherwise.

Note that, with a single possible exception, the X-ray fluxes implied by the temperatures and areas in Table 2 are all well below the sensitivities of previous X-ray sky surveys (The sensitivities of such surveys have typically been of order 1 Uhuru flux unit (UFU) = 1.7×10^{-11} erg cm^{-2} s^{-1} in the 2–10 keV band.) The exception is 1929 + 10, for which the predicted 2–10 keV flux should have been only marginally detectable and for which the value of B_{12}/P^2 is close to the cutoff. There is therefore clearly no serious conflict between the predictions of the model and the existing data.

The final column of Table 2 shows the number of 0.1–5.0 keV X-ray counts in the Einstein Observatory IPC expected in a 2,000-s observation. The calculations were performed as described above assuming an interstellar neutral hydrogen density of 1 cm^{-3} and with A as in column 5. We have taken into account the change in the projected surface area of the polar cap as the star rotates and have averaged radiation from both poles of the star by integrating the contribution from a random distribution of angles between the magnetic pole and the rotation axis. Clearly at least half of the sources would be easily detectable in such short observations. Even if the conversion efficiency is as low as $\eta \sim 0.01$, several objects could still be detected in 10^4-s pointings. Such observations will thus apply a stringent test to this or any similar models for γ-ray production in radio pulsars.

Pulsars with values of B_{12}/P^2 below the cutoff of ~ 10 still produce X-ray emission. In the inner-gap models of Ruderman and Sutherland[29], the predicted X-ray luminosity is given by

$$L_x \approx 1\times10^{30}\frac{\varepsilon\cdot B_{12}}{P^2}\ \text{erg s}^{-1} \tag{6}$$

where ε is related to the binding energies of the atoms in the crust of the polar cap ($10^{-3} < \varepsilon < 1$). Thus, expected X-ray luminosities range from $\sim 10^{32}\ \varepsilon$ erg s^{-1} for a 0.25-s pulsar with $B \sim 5\times10^{12}$ G down to $5\times10^{29}\ \varepsilon$ erg s^{-1} for a 1-s source with a field of 5×10^{11} G.

Assuming a black body spectrum for the radiation, we expect a temperature

$$T = \left(\frac{L_x}{A\sigma}\right)^{1/4} = 0.65\times10^6\left(\frac{L_x}{10^{28}\ \text{erg s}^{-1}}\right)^{1/4}\left(\frac{A}{10^9\ \text{cm}^2}\right)^{-1/4}\ \text{K} \tag{7}$$

where σ is the Stefan–Boltzmann constant [5.67×10^{-5} erg cm^{-2} s^{-1} K^{-4}] and A is the surface area of the polar cap. For even the closest pulsars, previous X-ray results are no help in setting a limit on ε: for PSR0950 + 08 with $P = 0.25$ s, $B = 5\times10^{12}$, and $D = 110$ pc, the flux is <1 UFU for $\varepsilon < 0.5$, and for all other pulsars, $\varepsilon = 1$ is allowed. Theoretical arguments (M. A. Ruderman, personal communication) suggest values for ε of $\sim 1/500$ for the fastest pulsars ($P < 0.1$ s) but allow ε to approach unity as the star ages.

The model of Arons and Scharlemann[33] predicts the lowest X-ray flux of any of the polar cap models suggested thus far. The total X-ray luminosity L_x is given by

$$L_x \approx 2\times10^{30}\left(\frac{0.1}{P}\right)^{27/8}\ \text{erg s}^{-1} \tag{8}$$

Thus, for this model, predicted luminosities range from 3×10^{30} erg s^{-1} for the Vela pulsar to 10^{28} erg s^{-1} and 8×10^{26} erg s^{-1} for stars with 0.5-s and 1.0-s periods, respectively. The implied temperatures fall between 0.4 and 2×10^6 K. As recently noted by Arons[35], equation (8) provides a lower limit to polar cap heating in the context of pair production models. The heating could be greater due to trapping of positrons in a region above the pair creation zone[33] or because of unsteady spark discharges as in the Ruderman and Sutherland models.

Table 3 lists the expected Einstein Observatory IPC counts in a 10^4-s observation for a range of polar cap temperatures. A polar cap area of 10^{10} cm^2 and an interstellar neutral hydrogen density of 1 cm^{-3} have been assumed. Setting our detection threshold at 25 counts, we see that only relatively nearby ($\lesssim$1 kpc) sources can be observed, primarily because these low energy X rays (10^6 K ~ 0.1 keV) are absorbed by the interstellar medium. From a compilation by J. H. Taylor (personal communication) of 325 known pulsars, we can expect only marginal detections under the assumptions of the Arons and Scharlemann model[33]. For the Ruderman and Sutherland theory, on the other hand, we can expect up to 20 strong detections, depending on the value of the parameter ε.

Dynamical friction: The widely quoted 'two-component' model of neutron star structure[38] suggests a neutron superfluid interior surrounded and threaded by a charged component tied to the star's magnetic field. The pulsar's deceleration torque forces the charged component to corotate with the field but does not directly affect the neutral superfluid. On average, then, this latter component is always rotating faster than the exterior, and the frictional coupling between the two components will dissipate some of the star's rotational kinetic energy as heat. Greenstein[39], building on the work of Ruderman and Sutherland[40] who demonstrated that a deceleration of differentially rotating superfluid may not tangle the superfluid vortex lines, has generated a self-consistent model for the temperature of a neutron star as a function of time which includes frictional heating. More recent work[41], including a careful treatment of relevant neutron scattering processes (by phonons, electrons and impurities), predicts that the temperature of a 0.5 $M_\odot$ neutron star will remain above 3.5×10^5 K for 10^7 yr. More massive stars maintain even higher temperatures (for $M = 1.5\,M_\odot$, $T \sim 5 \times 10^5$ K).

Independent lines of reasoning[42–45] lead us to believe that virtually all radio pulsars are $<10^7$ yr old. Figure 1 indicates then that, by using the Einstein Observatory, we may put the frictional heating results to a stringent observational test. The fraction of the stellar material in the superfluid state and thus the predicted temperatures are a strong function of stellar mass. If we accept the theory and yet detect no sources, the results may be interpreted as an upper limit on the masses of the various neutron stars involved. Alternatively, temperature upper limits below those predicted will provide information on neutron star structure. For example, the assumption that the superfluid vortex lines do not pin to nuclei in the crust[46] may have to be relaxed, implying that several other interesting phenomena must be considered[47–49]. Another possibility[41] is that neutron stars have larger radii than those derived from any of the currently employed equations of state. Note that, in this regard, if one measures both the luminosity and the temperature (by fitting a black body spectrum) of a neutron star, the radius—a critically important parameter for distinguishing among stellar models—is uniquely determined.

Starquakes: In addition to the potential thermal energy stored in the differentially rotating neutron star, a large amount of elastic and gravitational energy resides in the solid outer crust. The crust is distorted into an oblate spheroid by its rapid rotation. As the rotation rate decreases, the star tries to readjust, but the immense strength of the crustal material (sheer modulus $\mu \sim 10^{29}$ dyn cm^{-2}) resists, until the stress exceeds a critical value and the crust cracks. The resulting starquake was first proposed by Ruderman[50] to account for the sudden speed-up of the Vela pulsar. Although the magnitude of these 'glitches' ($\Delta P/P \sim 10^{-6}$) coupled with their roughly triennial recurrence casts serious doubt on this application of the starquake theory, it has been shown that erratic behaviour is a hallmark of pulsar rotation rates. The Crab pulsar has undergone at least two such discrete spin-ups (although of a much smaller magnitude: $\Delta P/P \sim 10^{-9}$), an older pulsar, PSR1641 − 45, has experienced a Vela-sized glitch, and a 'restless' wandering of rotation rates (often designated as 'timing noise') has been found in a large number of other objects (refs 51 and 52 summarise recent work). A starquake model may well be applicable to many of these phenomena[53].

Table 3 IPC counts from pulsar polar caps at distance D from Earth

Luminosity (erg s^{-1})	T (10^6 K)	Counts*			
		$D = 0.1$ kpc	0.3 kpc	1 kpc	3 kpc
10^{28}	0.36	1.5	0.015	—	—
10^{29}	0.65	56	0.68	0.0024	—
10^{30}	1.24	720	26	0.68	0.011
10^{31}	2.0	6,600	470	22	0.74
10^{32}	3.6	80,000	7,600	480	28

* Counts observable in a 10^4-s IPC pointing assuming an area for the polar cap of 10^{10} cm^2 and an interstellar neutral hydrogen density of 1 cm^{-3}.

The energy released in a typical starquake is of the order of 10^{40}–10^{41} erg (ref. 54). In the simple two-component picture described above, one can calculate the interval between such quakes $t_{\rm sq}$ from the measured values of the star's angular frequency Ω, frequency derivative $\dot{\Omega}$, change in frequency as a result of the glitch $\Delta\Omega$, and several parameters which describe the structural properties of the star[54,55]:

$$t_{\rm sq} = \left(\frac{2A^2}{BI}\right)\left(\frac{\Delta\Omega}{\Omega^2\dot{\Omega}}\right) \tag{9}$$

A is the gravitational energy stored in the star by virtue of its rotation, B measures the elastic energy stored in the solid crust due to its distortion, and I is the star's moment of inertia. While these parameters are quite sensitive to assumptions about the stellar mass and crustal properties, expected time scales range from ~10^4 yr for a 1.5 $M_\odot$ star to a few days for stars one-tenth as massive[55]. The observed interval for both the Vela and Crab pulsars is a few years. For older pulsars, only one large glitch has occurred in ~200 pulsar-years of accumulated data, although events with $\Delta P/P \sim 10^{-10}$–10^{-12} may be occurring frequently (hours to weeks) and manifesting themselves as timing noise[53].

This seismic activity results in an average rate of energy input to the neutron star $L_{\rm sq} \sim E_{\rm sq} t_{\rm sq}^{-1}$. If a large fraction of this energy is ultimately radiated away as thermal photons, the time-averaged luminosity will be ~10^{33} erg s^{-1} for both Crab-type glitches and the more frequent, smaller quakes which result in timing noise. Since the thermal time constant for the star is long relative to $t_{\rm sq}$, this process could maintain the stellar surface at temperatures of ~10^6 K for as long as it operates. Many (~50) nearby pulsars (see Fig. 1) would then be observable with the Einstein Observatory. Greenstein[56] has noted that the deposition of energy in a neutron star following a quake may trigger a thermal instability. By extracting energy from the more rapidly rotating interior, the star's temperature may rise significantly, rendering even more distant objects easily observable for some time after the event.

Accretion: It is beyond the scope of this article to discuss the thermal properties of neutron stars in binary X-ray systems where the dominant source of the observed radiation is material accreted from a normal stellar companion. However, we include a note on temperatures expected from an isolated star accreting interstellar material. If current estimates of the pulsar birthrate (~10^{-1} yr^{-1}) (ref. 42) are even approximately correct and if this rate has persisted throughout most of the life of the Galaxy, there are at present a large number of neutron stars (~10^9 or ~1% of the total galactic mass) which are no longer active pulsars. The implied space density is such that the nearest object is expected to be ~10 pc from the Sun. The accretion rate $\dot{M}$ for such a star with mass M travelling at velocity v through the interstellar medium of density ρ was first given by Bondi and Hoyle[57]:

$$\dot{M} = 3.73 \times 10^8 \left(\frac{\rho}{1\ \text{cm}^{-3}}\right)\left(\frac{M}{M_\odot}\right)^2\left(\frac{v}{100\ \text{km s}^{-1}}\right)^{-3} \tag{10}$$

for spherically symmetric accretion. (The very old, slowly rotating stars under consideration here will most likely have little or

Table 4 Hot neutron stars in supernova remnants

Name	Age (yr)	D (kpc)	$R_{\max}$* (arcmin)	T (10^6 K)	Ref.
Cas A	~300	2.8	0.4	<1.5	71
Kepler	375	8.0†	0.2	<2.1	Present work
Tycho	407	3.0†	0.5	<1.8	Present work
Crab	925	2.0	1.6	<3.0	10
SN1006	973	1.0	3.3	<1.0	F. D. Seward and J. P. Pye (personal communication)
RCW86 (AD185)	1,794	2.5†	2.5	<1.5	Present work
W28	3,400	2.3‡	5.0	<1.5	Present work
G350.0 −18	~10^4	4.0‡	7.0	<1.5	Present work
G22.7−0.2	~10^4	4.8‡	7.0	<1.8	Present work
Vela	~10^4	0.4	1.4	~1.5	72

* Distance from SNR centre assuming a transverse velocity of 1,000 km s^{-1}.
† Ref. 69. ‡ Ref. 70.

no relativistic outflow to resist the accretion, although a significant remnant magnetic field may channel the material onto the polar cap regions.) Measured pulsar velocities range from 20 to 500 km s^{-1} (refs 58–62). Adopting a characteristic conversion efficiency of rest mass energy of the accreted material to X rays of 0.1, we find a luminosity of 4.5×10^{29} erg s^{-1} for a 1.3 $M_\odot$ neutron star travelling at 50 km s^{-1} through a medium with $n_H = 1$ cm^{-3}. Such a star would be detectable to a distance of ~50 pc and might be distinguished from other background X-ray sources without optical counterparts by its large proper motion of ~1 arc s yr^{-1}. Stars passing through regions of much higher density (molecular clouds with $n_H \sim 10^6$) will have proportionally higher luminosities, although the increased absorption in such a region will probably render them undetectable.

Thermal dissipation—general considerations: Greenstein and McClintock[5] pointed out that one might expect heating of neutron stars on general grounds from thermal dissipation of rotational energy. We consider here one circumstance under which such dissipation might take place, and then look at the size of the dissipation effects which could be measured in radio pulsars using the Einstein Observatory.

From the expressions for the rotational energy ($E = \frac{1}{2}I\Omega^2$) and angular momentum ($J = I\Omega$) of a rigid body, we have

$$\Delta E = \Omega\Delta J \tag{11}$$

This specifies a relationship between the energy lost and angular momentum lost by a spinning neutron star as it slows down. Now a photon of energy $E_p = \hbar\omega$ emitted tangentially from the light cylinder (at radius $r = c/\Omega$ from the neutron star) will carry off an amount of angular moment J_p given by

$$J_p = \frac{\hbar\omega}{c}r = \frac{\hbar\omega}{\Omega} \tag{12}$$

so that

$$E_p = \Omega J_p \tag{13}$$

that is, radiation from the light cylinder carries off just the right amount of energy per unit angular momentum to satisfy equation (11); there is none left to heat the star. However, this situation is idealised in that it assumes corotation of the neutron star magnetosphere. In some circumstances (when $\mathbf{E}\cdot\mathbf{B}\neq0$ along the field lines), Ω in fact will decrease outwards, causing the angular velocity Ω_{lc} at the light cylinder to be less than the neutron star angular velocity Ω. In this case, we have

$$E_p = \Omega_{lc}J_p < \Omega J_p \tag{14}$$

and the photons do not carry off sufficient energy per unit angular momentum; there is an excess $E_p - \Omega J_p$ per photon which is available to heat the star.

We can characterise such thermal dissipation from a neutron star in terms of the ratio $\eta = L_x/\dot{E}$ of the (thermal) X-ray luminosity to the rotational energy loss rate. Here η is not an efficiency in the usual sense (it could greatly exceed unity, for example, in the case of a hot young star). However, for old neutron stars which have had sufficient time to cool, η does provide a measure of (or upper limit on) the thermal dissipation of rotational energy.

For pulsars with measured period derivatives, one can calculate $\dot{E}$ using theoretical estimates of the moment of inertia[26]. The results generally lie in the range 10^{31}–10^{33} erg s^{-1}. Figure 1 shows that within ~100 pc, neutron star temperatures as low as 250,000 K, corresponding to luminosities of ~10^{31} erg s^{-1}, can be detected with the IPC. Thus in several cases one could establish limits to η of ≲1%. That is, a fairly small fractional thermal dissipation of the rotational energy is, in principle, detectable. Such observations can therefore be expected to provide important constraints for the construction of pulsar models.

Observations

Past results: The observational situation regarding thermal emission from neutron stars has not been as rich in scope or detail as the theoretical work described above. The first attempt to measure the temperature of a neutron star was carried out in 1963 (before the discovery of pulsars) when a group from the Naval Research Laboratory observed a lunar occultation of the Crab Nebula with a rocket-borne X-ray detector[63]. The result showed that the bulk of the X-radiation from the Crab came from a spatially extended source, implying that the temperature of any associated hot neutron star must be $\lesssim10^7$ K. During the next series of Crab occultations, 11 yr later, two groups flew improved detectors and set limits to the temperature of the now-confirmed neutron star in the Nebula of 4.7×10^6 K and ~3×10^6 K, respectively[10,17]. Poor spatial resolution has meant that observations of other SNR with both rocket and satellite experiments have failed to yield significant ($T < 10^7$ K) upper limits for any other young objects. Limits on the temperatures of older neutron stars (radio pulsars) have been calculated using data from the Uhuru all-sky survey[5]; the results for the nearer pulsars ($D < 500$ pc) lie in the range 2.0 to 3.0×10^6 K, far above most of the predictions summarised above. Similar studies using data from Copernicus[64] and ANS[65] have yielded slightly higher limits. A recent optical spectrographic search of several historical SNR[66] showed no evidence of peculiar objects in the central regions of the nebulae, but the limiting magnitude of the survey was m_{pg} 18.5 (see Table 1). Finally, the best limits on neutron star temperatures recorded before this year were obtained with the extreme UV telescope aboard the Apollo–Soyuz mission[67]. The results for three nearby pulsars ranged from 0.5 to 5×10^6 K but are extremely sensitive to the values assumed for the photoelectric opacity of the interstellar gas.

The Einstein Programme: The observing program of the Einstein Observatory calls for observations of over 50 SNR and 35 radio pulsars. All the historical SNR will be studied extensively with both the IPC and the high resolution imager (HRI—spatial resolution of 5 arc s)[18]; over a dozen older remnants within 3 kpc will also be examined closely for hot neutron star remnants. The pulsar survey includes many objects selected on the basis of short periods, large values of B_{12}/P^2, and large amplitude timing noise to provide the most stringent tests possible for the various theories of pulsar emission mechanisms and neutron star structure discussed above. We have also included an unbiased survey of all objects within 350 pc of the Earth. We present below the first results of these observations.

Supernova remnants: Table 4 lists results for 10 of the SNR observed with the Einstein Observatory. The objects are ordered by age with the six historical remnants heading the list; for the remaining objects we have adopted ages determined by application of the standard blast wave (Sedov) model to the X-ray data[68]. The distances adopted are published values and are consistent with those found from the measured hydrogen

Table 5 Temperature limits for radio pulsars

Name (PSR)	P (s)	$\dot{P}$ (10^{-15} s s^{-1})	D (pc)	Counts (0.1–5.0 keV)	$T_{n_0=0.3\ \mathrm{cm}^{-3}}$ (10^6 K)	$T_{n_0=1.0\ \mathrm{cm}^{-3}}$ (10^6 K)	T_{cap}* (10^6 K)	L_{cap} (10^{30} erg s^{-1})
0031−07	0.943	0.40	440	26	0.4	0.7	2.4	5.5
0149−16	0.833	—	560	19	0.5	0.9	2.7	9.8
1529+28	1.125	—	640	16	0.5	0.9	2.9	9.6
1642−03	0.388	1.8	160	18	0.3	0.35	1.2	0.8
1706−16	0.653	6.4	160	29	0.3	0.4	1.4	0.9
1952+29	0.427	0.002	240	31	0.3	0.4	1.5	1.8
2327−20	1.644	—	320	17	0.4	0.5	2.2	2.2

* Polar cap areas have been calculated using equation (5) and assuming a radius of 16 km. A hydrogen density of 1 cm^{-3} has been adopted. Changing either of these assumptions by a factor of 2 changes the derived temperature limits by less than 30%. All temperatures and luminosities listed are 3σ upper limits.

column density derived from the X-ray spectra assuming a reasonable range of average interstellar values of n_{H}. (For example, the radio absorption spectrum[73] and X-ray determined column density of Tycho's remant imply a neutral hydrogen density of $\sim$0.5 cm^{-3} for the adopted distance of 3 kpc.) Table 4 also lists the distance in arc min from the centre of the explosion within which a neutron star would be found if it had a transverse velocity of <1,000 km s^{-1}. (The mean velocity of radio pulsars derived from proper motion measurements is $\sim$150 km s^{-1} and the highest observed velocity from a sample of 25 objects is 500 km s^{-1} (ref. 62).) The temperature upper limits for a point source of X rays in these remnants is also given. For the Columbia IPC observations, we have determined a 3σ upper limit to a point source counting rate by sliding a 2 arc min radius circular detection cell through the allowed region and determining the local background level from a concentric annulus with inner and outer radii of 2 and 3 arc min, respectively. This value is then converted to a temperature limit following the procedure and adopting the assumptions outlined above. For Kepler's remnant, preliminary HRI data have recently become available, and we have used these with a 12 arc s search cell to set an upper limit. Similar procedures were used for the remaining observations (see cited refs for details).

Of the six historical SNR, only the Crab is known to contain a remnant neutron star. The analysis of Einstein data for this source is not yet complete, so its temperature limit remains at the value from the lunar occultation experiments. For all of the remaining historical objects, the measured temperature limits fall below the lower limits predicted by nearly all current work on the cooling of neutron stars.

Among the four older sources, only Vela was known to contain a neutron star, and again the situation remains unchanged. The limits on the remaining three sources are also either below or just consistent with the lower bounds derived from standard cooling theories. However, a point source has tentatively been detected at the position of the Vela pulsar[73], and the derived temperature of 1.5×10^6 K is consistent with that expected for a 10^4 yr old star. The observation is somewhat complicated by the fact that the point source is surrounded by a small (1 arc min) region of diffuse emission and the interpretation of the central cusp of this nebula as thermal radiation from the neutron star should be treated with caution.

Radio pulsars: Seven nearby radio pulsars have been observed in the Columbia survey; the sources are listed in Table 5. No sources have been detected at the nominal pulsar positions. Upper limits to the IPC counting rates and the resultant temperature limits for thermal stellar emission were derived as discussed above. Limits on polar cap temperatures and total luminosities are also given. All of these values are typically an order of magnitude below those previously available and provide interesting constraints on several theoretical problems.

Discussion

An observational determination of the cooling rate for young neutron stars is a fundamental input to models of neutron star structure. Such a determination is within the capabilities of the Einstein Observatory. The results on the historical SNR imply either that many contain no neutron star remnant, or, in the context of our current understanding of cooling theory, pion condensation may need to be invoked in order to enhance the cooling rates. In view of the importance of the latter possibility, we wish to encourage thorough theoretical studies of neutron star cooling to verify this conclusion.

The option that some supernovae leave no neutron star remnant also holds important implications. While supernova models which produce neutron stars have recently been constructed, the alternatives of a black hole remnant or an explosion which dissipates the entire star will have to be explored. A more immediate problem, however, concerns the pulsar birthrate. The current best estimates for the galactic supernova rate is one per 30–50 yr. The pulsar birthrate has been estimated to be one per 5 yr (ref. 42). This latter value is sensitive to several assumptions, such as the electron density of the interstellar medium which sets the pulsar distance scale and the 'beaming factor' which specifies what fraction of the total population is observable. Taylor and Manchester[42] assume a pencil beam radiation pattern for pulsars and adopt a beaming factor of 20%. If, in fact, the pattern were closer to a fan beam such that essentially all pulsars were observable, much of the above-noted discrepancy would disappear. The fact that only one of the five supernovae of the past 1,000 yr has left behind an observable radio pulsar has been used as an argument in favour of the pencil beam model. However, the lack of an X-ray point source in the remaining objects may suggest that only a small fraction of SN events leave neutron star remnants and, thus, even if a fan beam model is adopted, the discrepancy between supernova and pulsar production rates will remain large. Some alternative production mechanism for neutron stars, such as accretion pushing a white dwarf over the Chandrasekhar limit, may have to be considered.

While the observation of newly formed neutron stars in supernova remnants promises to yield valuable information for our understanding of neutron star matter, X-ray data on the thermal properties of radio pulsars will provide constraints on models for many aspects of pulsar behaviour, including crust–core coupling, starquakes, pulsar emission mechanisms, and so on. Our preliminary results illustrate the model testing that such observations may provide, even without the actual detection of thermal radiation. In testing polar cap emission models we have calculated the expected luminosities based on equations (5)–(8); for sources without measured values of $\dot{P}$ (and, thus, no computed value for the field strength), we have assumed $B_{12} = 1$. While none of these objects is expected to exhibit the intense radiation characteristic of the γ-ray emitting pulsars, three of the sources, PSR1642−03, PSR1706−16 and PSR1952+29 constrain the value of ε in the inner gap theory[29] to be $\varepsilon \lesssim 0.1$ (the limits are still above the predictions from the minimum flux polar cap model of Arons and Scharlemann[33]). Using the frictional heating model[40], we can set mass limits on the three sources with temperatures of <300,000 K of $<0.5\,M_{\odot}$, significantly below the measured masses of neutron stars in binary systems. Alternatively, in a model-independent way, we can say that for PSR1642 − 03 and PSR1706 − 16, the efficiency for conversion of rotational to thermal energy is <1%. More results will make all these constraints much more definitive as we open a new window for observational studies of neutron stars through the search for their thermal X-ray emission.

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ARTICLES

Reactivation of basement faults and crustal shortening in orogenic belts

J. A. Jackson

Department of Geodesy and Geophysics, Madingley Rise, Madingley Road, Cambridge, UK

During the early evolution of some fold mountain belts old listric normal faults in a stretched and thinned basement beneath the sedimentary column may become reactivated as thrust faults. The reversal of motion on these faults allows considerable shortening to occur without subduction or excessive thickening of continental crust. This reactivation hypothesis is supported by the present-day seismicity of the Zagros collision zone and the structure of sedimentary basins.

PLATE tectonics has been very successful in describing the overall deformation and kinematics of the large ocean basins but there is no comparably simple description to account for the general behaviour of the continents.

Recent studies of continental deformation have indicated some of the notable differences between oceanic and continental behaviour. The major strike-slip faults in the Alpine–Himalayan Belt[1-3] seem to slide large wedges of continental material away from zones of collision, thereby avoiding the excessive thickening of continental crust. Such large strike-slip features are easily visible on satellite photographs, and their spectacular nature, together with various models proposed to explain their large-scale significance[2-5], have to some extent diverted attention from the large areas of the Alpine–Himalayan belt which are undergoing active compression or extension.

These studies show that present day continental deformation is not a simple phenomenon. The deformation was unlikely to be less complicated in the past, and models for the evolution of old fold mountain belts based on oceanic compressive margins probably have limited value. In particular, little progress seems to have been made towards solving the classical space problem in orogenic belts: if the upper crust is shortened several tens of kilometres by folding and thrusting, what happens to the basement? Very often the sediments involved in foreland folding and thrusting are separated from their basement by a decoupling horizon, often of evaporites or shale, which provides a surface of décollment separating structures above from those below. Subduction has been discussed as a mechanism for removing continental crust[6,7], and it has been concluded that the buoyancy of continental material should hinder its subduction and lead to crustal thickening instead[7,8].

Sedimentary basins

Helwig[9] pointed out how this space problem in shortened orogenic belts is greatly diminished if the basement underneath the folded belt is thin to start with. Subsequent shortening then

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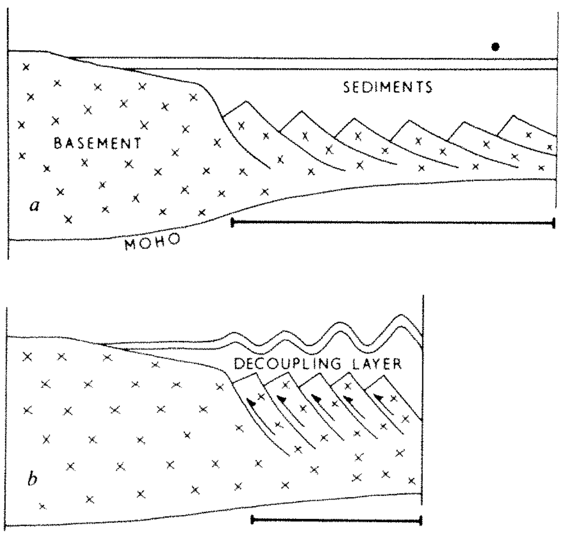

Fig. 1 Reactivation of basement faults. *a*, Sedimentary basin formed by deposition on a basement which has been stretched and thinned by listric normal faulting. *b*, When the basin contracts motion is reversed on the normal faults which are now used as thrusts. The sedimentary column takes up the shortening by folding.

thickens it, but not to an abnormal degree. McKenzie[10] has since shown that the subsidence of continental sedimentary basins can be quantitatively described by a simple model in which the lithosphere is rapidly stretched and thinned, sinks quickly to maintain isostatic equilibrium, and then sinks more slowly as it cools in a manner similar to that described by the successful oceanic models[11]. The initial stretching of the basement at shallow depths is thought to occur by listric normal faulting and thereafter stretching ceases and subsidence continues without further faulting. This model is supported by the crustal thickness and heat flow of the Aegean, which is still being stretched by normal faulting, and seems to account well for the subsidence history of the North Sea[12] and many of the intra-Carpathian basins[13], all of which show an initial normal faulting episode followed by subsidence with no further faulting. Royden *et al.*[14] have shown that the gross features of the same model will account for the stretching and subsidence preceding the break-up of a continent to form an Atlantic-type margin. It thus seems probable that thick sedimentary sequences generally form on thin extended basement which was stretched by normal faulting. Evidence for attenuated basement is best seen in seismic refraction profiles and has been demonstrated in the North Sea[15,16] and Pannonian Basin[13]. The Aegean is in an earlier stage of development, with high heat flow[17], thin crust[18,19] and active normal faulting[20]. Helwig's[9] suggestion may, therefore, have some validity and the basement under the thick piles of sediments that make up fold mountain belts was probably thin before the onset of compression. The early stages in the development of a fold mountain belt following continental collision are now considered.

It is proposed that thick sediments were initially deposited on a subsiding basement which had been stretched by normal faulting (Fig. 1). The faulting was very probably of a low angle listric nature, spread over a region several tens of kilometres wide as observed in the Great Basin[21]. As stretching proceeded, an Atlantic-type margin was ultimately formed with thick sediments on the continental shelf overlying a thin basement. In the early stages of continental collision the basement takes up the shortening by reversing the sense of motion on the pre-existing normal faults (Fig. 1), which are now used as thrusts (reverse faults). The basement tends to return to its original thickness while the overlying sedimentary column, having been deposited on an extended basement, is forced to take up the shortening by folding. Thus in the early stages of collision the space problem is avoided without thrusting continental basement into the mantle or thickening it beyond its original (unstretched) state.

Seismicity of the Zagros mountains, Iran

The Zagros mountains of Iran provide an example of a young fold mountain belt currently shortening as a result of the collision between Arabia and Iran. The structure of the Zagros is very simple superficially, with a thick conformable sequence of Palaeozoic–Mesozoic–Tertiary shelf deposits warped into gentle folds in a single process in the latest Tertiary[22–24]. Long linear fold axes and seismic activity that is predominantly thrusting of the same orientation (refs 1, 25 and Fig. 2) both indicate a general NE–SW shortening. Although strike-slip faulting is present on the north-east front of the Zagros, especially in the north[26,27], it is not seen in the fault plane solutions of the folded belt (Fig. 2) and the relative motion between Arabia and Iran is normal to the strike of the belt.

The Folded Belt[22] of the Zagros is seismically very active with earthquakes spread over an area ∼300 km wide and with a distinct northeastern boundary roughly coincidental with the Zagros Thrust Line (or Main Zagros Reverse Fault, Fig. 2 and ref. 27), which in turn marks the edge of the Zagros sedimentary trough. These earthquakes are probably all shallower than 40 km (see ref. 28 for discussion) and there is no reliable evidence that they increase in depth towards the north-east margin of the Zagros. Consequently there is no evidence for seismic shortening on a single shallow-dipping plane, as is commonly assumed for areas of oceanic underthrusting. Fault plane solutions in the Zagros consistently show thrusting with comparatively high angle (40–50°) fault planes (ref. 29 and Figs 2 and 3). This is in marked contrast to the shallow angle (0–10°) thrusting which occurs in areas where oceanic underthrusting of continents is or was taking place, such as the Hellenic Arc, Makran and Eastern Himalaya[29]. The seismic activity of the Zagros Fold Belt reflects high angle reverse faulting on a large number of faults distributed across the whole width of the belt.

Although microearthquake surveys in the Zagros have shown that small earthquakes do occur within the sedimentary column[30–32] it has been suggested that the largest shocks, which reach m_b 6.0–6.3, have focal depths of about 15 km, that is, beneath the sedimentary cover and in the crystalline basement. Berberian and others[27,33] reached this conclusion from a study of damage distributions and because of the lack of surface faulting

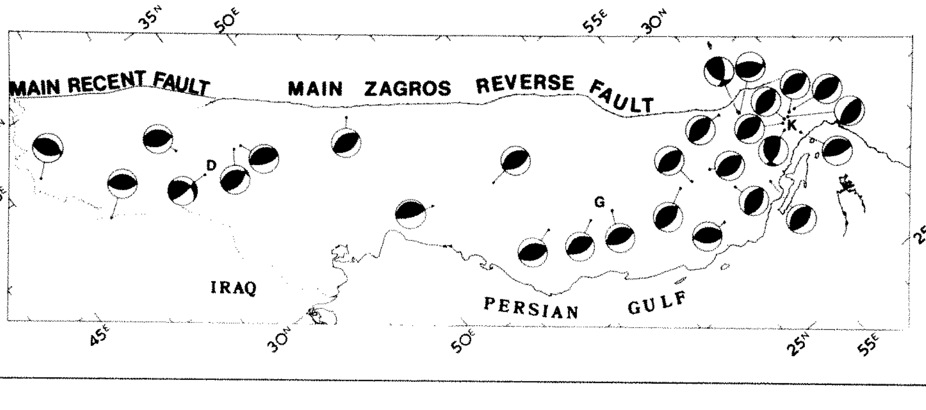

Fig. 2 Fault plane solutions from the Zagros from ref. 25, to illustrate the high angle thrusting in the Folded Belt south-west of the Main Zagros Reverse Fault (called the Zagros Thrust Line by Stocklin[24] and others). D, G and K mark the positions of the Dezful Embayment, Ghir and Khurgu earthquakes. Black quadrants are compressional.

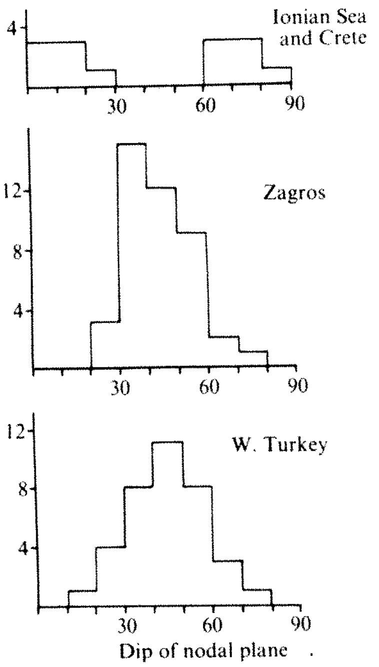

Fig. 3 Histogram to illustrate the high angle (40–50°) nature of the fault planes in the Zagros compared with those in the Ionian Sea and Crete, which are both part of the Hellenic arc where active subduction (intermediate depth earthquakes and andesitic volcanism) is taking place and thrusting is on shallow dipping (0–10°) fault planes. The Zagros histogram is similar to that of western Turkey where active extension by normal faulting is occurring. Dips are taken from the fault plane solutions in refs 1, 20, 25 and are mostly well controlled in the Zagros, even though the strikes may not be.

in the Zagros after major earthquakes. At the base of the sedimentary column is the thick infracambrian Hormuz Salt Formation, and additional salt is present in the Jurassic and especially in the Tertiary Gach Saran Formation[23]. Any basement faulting is unlikely to propagate to the surface through these ductile horizons. It is also unlikely that shocks the size of the 1972 Ghir earthquake ($m_b = 6.0$), with a source dimension of about 30 km (refs 34, 35), could be contained within 6 km of sediment thickness without showing surface faulting. The most convincing evidence of basement faulting comes from detailed examination of the long period teleseismic waveforms from Zagros earthquakes. Shocks in the magnitude range m_b 5.5–6.0 are common in the Zagros, and their far-field long period radiation is usually very simple. In particular, the first cycle of the waveform can easily be modelled and is very dependent on focal depth. At long periods crustal structure has little effect on the first cycle of the waveform if the source is above the Moho, and earthquakes of this size can be modelled effectively by a single source as they rarely exhibit multiple rupture characteristics. Studies of the waveforms[36] demonstrate that in three areas of the Zagros—the Dezful Embayment, Ghir and Khurgu (Fig. 2)—earthquakes occurred at depths of 12–15 km. In each case this is below the probable thickness of the sediments[37–39]. An example of this waveform modelling is shown in Figs 4 and 5. There is, therefore, the strong probability that the basement beneath the Zagros Fold Belt is deforming by high angle (40–50°) reverse faulting.

Evolution of the Zagros

There is much evidence that, during the Mesozoic and Tertiary, what is now the folded belt of the Zagros was a subsiding continental margin[23,24,40,41]. As the basement was almost certainly cut by normal faults which caused its extension before the onset of subsidence and sediment deposition in the Permo-Triassic, the present day reverse faulting is probably happening on the old normal fault surfaces which have been reactivated. This would explain the high (40–50°) rather than low (0–10°) angle nature of the fault dips, and the similarity of the dips of fault planes in western Turkey (currently extending by normal faulting) with those in the Zagros (Fig. 3). Reactivation of old faults is a common and important phenomenon[42] and the reactivation of old normal basement faults as thrusts has been reported from Chile[43]. The possibility of reactivation in the Zagros was alluded to by Falcon[22], and Stocklin[24] proposed that the Zagros Thrust Line itself was a reactivation of an old Precambrian rift boundary which controlled the distribution of the Hormuz salt. The north-west trend of the Zagros is found in the Precambrian of the north-east Arabian shield[24]. The same trend is evident in the sediment isopachs of the northern Zagros (Lurestan and Kuhzistan) throughout the Mesozoic[44,45].

An objection to this suggested basement involvement is the evidence from seismic reflection lines in old fold mountain belts, such as the eastern Rockies[46,47], which shows apparently undeformed Precambrian shield dipping gently beneath the foreland folds. However, it is likely that in these older orogenic belts the amount of shortening involved is considerably greater (~160 km, or 50%, in the Canadian Rockies[46,47] than that which has taken place so far in the Zagros Fold Belt (about 20–50 km, or 20%, ref. 22) which is in a much younger state of development. The contraction mechanism proposed here will presumably work until the reverse motion on old normal faults restores the basement at least to its original thickness. Thereafter the basement will either become thicker than its pre-stretched state or be thrust into the mantle. It is probable that the sediments of the folded belt of the Zagros are still almost above the original basement on which they were deposited. As shortening continues, the folded cover will start to migrate south-west over the undeformed Arabian shield, separated from its basement by the décollment at the level of the Hormuz salt. The foreland folds of the Rockies, Appalachians and Jura are no longer above their original basement which was left behind in the internal zones. Those orogenic belts which are known to have thick crust

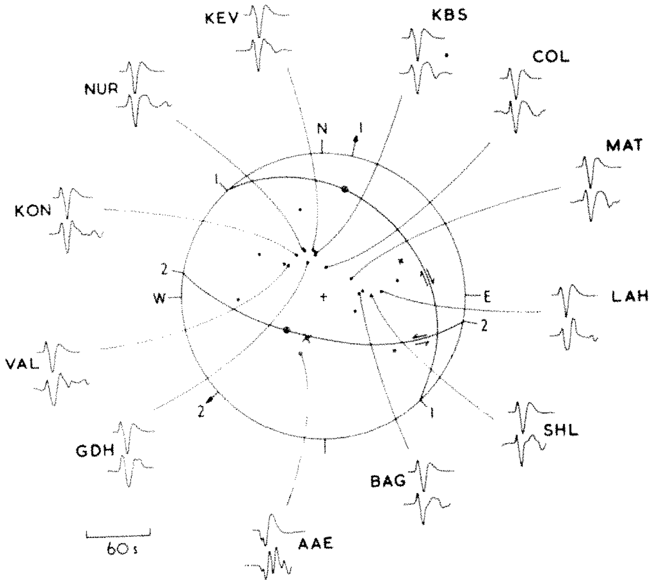

Fig. 4 Waveforms from a shock in the Dezful Embayment (Fig. 2) on 5 June 1977. The fault plane solution shows predominantly thrusting. Filled symbols are compressional first motions, open are dilatational and crossed are nodal. Synthetic waveforms matched the observed well at a focal depth of 12 ± 3 km (Fig. 5). For each station the observed seismogram is shown beneath the synthetic. Note particularly the kink in the downward pulse at AAE which is the pP arrival. Numbered arrows mark the slip vectors corresponding to planes 1 and 2.

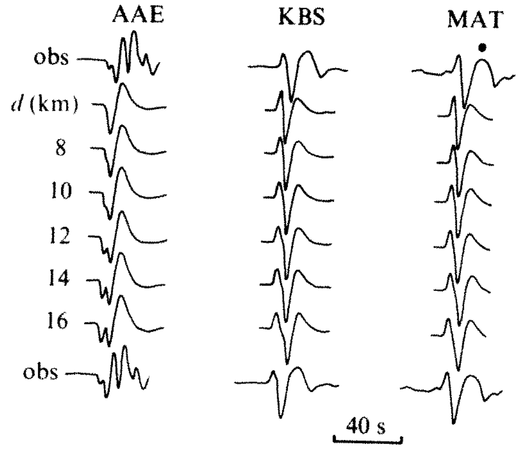

Fig. 5 Variation of waveform with depth (*d*) at selected stations in Fig. 4.

are all older and in more advanced states of development than the Zagros and have involved more shortening. An interesting prediction of the scheme proposed here is that, in the initial stages of continental collision, the basement under the orogenic belt should be thinner, not thicker than that of its neighbouring craton. At present no adequate refraction data from the Zagros is available to test this.

Conclusions

The origin of a young folded belt proposed here accounts well for the present-day seismicity and deformation of the Zagros. Such an origin involves the reactivation as thrusts of listric normal faults in a stretched and attenuated basement on which thick sediments of the Atlantic-margin type were deposited, and does not necessitate either abnormal thickening or subduction of continental crust in the early stages of continental collision. How long this process can continue is not clear. It is unlikely to account for all the features of older orogenic belts which have involved considerably greater shortening. However, even if operative in only the early stages of collision, it greatly reduces the space problem encountered in palinspastic reconstructions of orogenic belts[49].

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Lateral mobility in reconstituted membranes—comparisons with diffusion in polymers

Melvin Schindler* & M. J. Osborn
Department of Microbiology,

Dennis E. Koppel
Department of Biochemistry, UCONN Health Center, Farmington, Connecticut 06032

The diffusion coefficients (D) *of lipopolysaccharide, phospholipid, and* Escherichia coli *matrix protein were determined in reconstituted multibilayer membranes. Over a range of protein concentration of 0–60% by weight,* D *for lipopolysaccharide decreased 10-fold, whereas* D *for phospholipid remained essentially constant. The diffusion coefficient of matrix protein at a concentration of 50% was* $\leq 10^{-12}$ cm^2 s^{-1}*. These results are discussed in terms of a model for diffusion in polymeric networks.*

THE fluid dynamics of biological membranes has been clearly established with the emergence of physical techniques to determine quantitatively the diffusion characteristics of membrane components[1–4]. Measurements of diffusion constants by fluorescence photobleaching methods have provided evidence for the lateral mobility, over micrometre distances, of lipids, glycolipids and integral membrane proteins in eukaryotic cells (see, for example, refs 5–10). In addition, measurements of lipid and glycolipid diffusion have been obtained in phospholipid model membrane systems devoid of proteins[11,12]. To extend these results and provide a systematic approach to an understanding of membrane diffusion, we have developed a reconstituted membrane system containing an integral membrane protein. We believe that the techniques used can be

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* To whom reprint requests should be addressed.

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extended to reconstitute other membrane systems for mobility investigations.

The reconstituted system to be described is composed of matrix protein, phospholipids and lipopolysaccharide isolated from the outer membrane of Gram-negative bacteria, *Escherichia coli* and *Salmonella typhimurium*. The outer membrane contains approximately 20–25% phospholipid, 30% lipopolysaccharide and 45–50% protein[13]. The matrix protein of *E. coli* (molecular weight of monomer, 36,000) is a transmembrane protein which forms hydrophilic pores, allowing low molecular weight carbohydrates, amino acids and ions through the permeability barrier of the outer membrane[13]. In association with lipopolysaccharide, it forms a phage receptor[14]. Electron microscopy of *E. coli* membrane preparations has demonstrated that matrix protein forms a highly periodic monolayer consisting of homopolymer trimers interacting to cover the peptidoglycan surface[15,16]. Reconstituted in vesicles, it retains its pore function[17], can inactivate phage and forms voltage-dependent aqueous channels in planar lipid bilayers[18]. Lipopolysaccharide is an amphipathic glycolipid that is only found in the outer membrane of Gram-negative bacteria and seems to be a major contributor to the permeability barrier in these organisms[19].

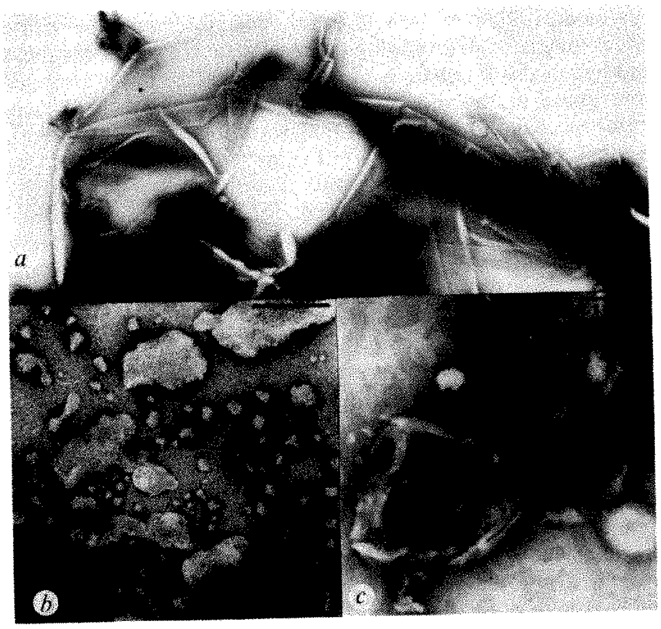

Fig. 2 Electron microscopy of vesicle preparations. Vesicles composed of phospholipid/matrix protein/lipopolysaccharide at ratios of 1:1.3:1 (*a*), 1:4:1 (*b*), and 1:0:1 (*c*), were negatively stained with 2% uranyl acetate on Formvar-coated specimen screens. Samples were examined with a Hitachi 11 E electron microscope at 75 kV. Scale bar, 250 nm.

The experiments to be described suggest that diffusion of membrane components in the plane of the membrane is analogous to the diffusion of non-electrolyte molecules in a polymer network. Lieb and Stein have presented such a comparison for diffusion *through* biological membranes[20,21]. Our results should enable us to extend their observations and make some general statements concerning two-dimensional diffusion in membranes.

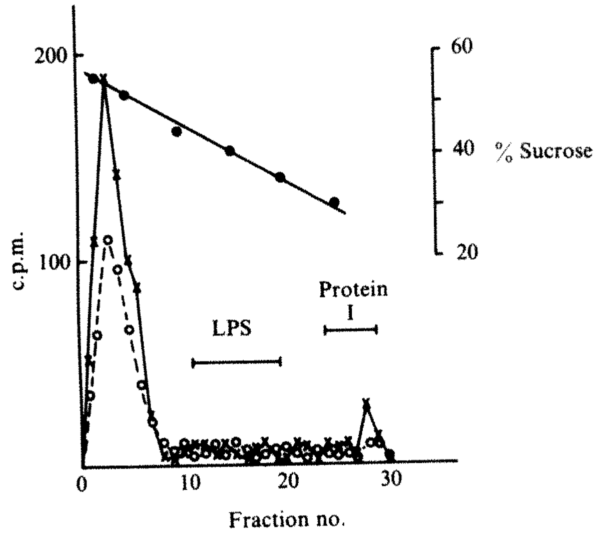

Fig. 1 Sucrose density gradient of reconstituted vesicle preparation (1:1.3:1, phospholipid/protein/lipopolysaccharide). Vesicles were prepared by the method of Nakae[17] with minor modifications. Phospholipids were isolated from *E. coli* K12 grown at 37 °C by the method of Raetz and Kennedy[34]. Lipopolysaccharide was derived from strain G30 of *Salmonella typhimurium* by the method of Galanos *et al.*[35]. Further purification was achieved by electrodialysis[36]. Matrix protein was isolated by the procedure of Hindennach and Henning[37]. The protein was then applied to a P-150 column (2.5 × 40 cm) water jacketed and eluted at 37 °C with 10 mM Tris, 2% SDS, 0.5 M NaCl and 5 mM EDTA to remove bound lipopolysaccharide. The protein was solubilised by slow dialysis of an 8 M urea–protein solution against 10 mM HEPES, *p*H 7.5. Protein concentrations were monitored by modified Lowry procedure and spectrophotometrically using an extinction coefficient, $A_{280}^{1\%} = 1.3$. Following an overnight incubation procedure[17], 20 mM disodium EDTA was added to the vesicle preparations, which were then placed on ice for 10 min and dialysed overnight at room temperature against 10 l of distilled water. Vesicles (50 µl) containing ^{3}H-labelled phospholipid (×) and ^{14}C-labelled lipopolysaccharide (○) were resuspended in 1 m of 25% sucrose (w/w) in 10 mM HEPES, *p*H 7.5, and layered onto a sucrose gradient containing 2.1 ml each of 50, 45, 40; 35 and 30% sucrose (w/w) over a cushion (0.5 ml) of 55% sucrose. All sucrose solutions contained 10 mM HEPES, *p*H 7.5. Centrifugation was carried out in a Beckman SW 41 rotor at 36,000 r.p.m. for 18 h at 4 °C. Gradients were fractionated by puncturing the bottom of the centrifuge tube and collecting drops. Protein was assayed by fluorescamine labelling and fluorimetric analysis of each gradient fraction.

Characterisation of vesicles and multibilayers

Isopycnic sucrose gradient centrifugation of vesicles (Fig. 1) showed a single homogeneous peak containing phospholipid lipopolysaccharide and protein. Figure 2*a* is a negative-stained preparation of the vesicles (phospholipid/protein/lipopolysaccharide, 1:1.3:1) before multibilayer formation. At protein concentrations of >20% by weight, long sausage-shaped sacks (~1 µm long) were the predominant vesicle species with a subpopulation of smaller vesicular structures which were probably intermediates between small unilamellar vesicles and the larger cylindrical vesicles. Figure 2*b* is a negative-stained preparation of vesicles (1:4:1) at higher resolution demonstrating ordered membranous lattice structures. These structures do not appear in protein-free vesicles (Fig. 2*c*). Comparison of Fig. 2*b* and *c* provides evidence that (1) the matrix protein is physically integrated into the membrane, as also indicated by the sucrose density gradients, and (2) the protein is uniformly distributed over the surface of the membrane. Figure 3*a*, *b* shows thin sections of multibilayer preparations from such vesicles. These sections indicate that stacked bilayers are formed from the vesicles and that these bilayers extend over long distances (approximately 1–2 µm in the figure).

Determination of diffusion coefficients

Lateral diffusion coefficients of fluorescently labelled phospholipid, lipopolysaccharide and protein were determined in 'fluorescence redistribution after photobleaching' experiments. In this technique (also referred to as 'fluorescence photobleaching recovery'), translational transport in the plane of the membrane is characterised by the kinetics of fluorescence 'recovery', due to the spatial redistribution of fluorescently labelled molecules after an initial, localised photobleaching pulse. A new multi-point analysis was used in which the fluorescence redistribution is monitored by a focused laser beam sequentially positioned at each of several locations immediately adjacent to the bleached area. The spatial information thus obtained provides sensitivity to possible systematic flow and a direct means of determining characteristic transport distances and hence absolute values of transport coefficients. A detailed description of the theory and experimental optics and electronics used in the multi-point analysis is presented elsewhere[22]. Briefly, a standard fluorescence microscope, equipped for incident light excitation, is used to focus the laser beam onto the sample and collect the fluorescence for detection by a thermoelectrically cooled photomultiplier tube. The precise orientation of the incident laser beam, and hence the location on the sample of the focused spot along a scan axis, is modulated by a servo-activated galvanometric optical scanning mirror. The laser intensity is switched between monitoring and bleaching levels, differing by a factor of 2×10^4, by a combination of diaphragms, beam-splitters and an electronic shutter.

N-4-nitrobenzo-2-oxa-1, 3-diazole (NBD) fluorescence was monitored with an incident wavelength of 4,765 Å and a combination of Leitz dichroic mirror TK510 and barrier filter K510. Rhodamine fluorescence was monitored with an incident wavelength of 5,309 Å and a combination of Leitz dichroic mirror TK580 and barrier filter K570. The membranes were examined before the photobleaching experiments with dark-field or phase-contrast transmitted light illumination. All photobleaching measurements were carried out with a 10×/0.25 NA dry achromat.

In most experiments, the fluorescence was monitored at three positions on a scan axis, with Δx, the position of the monitoring beam relative to the bleach position, equal to 0 and $\pm3.0\ \mu\text{m}$. Experimental values of $F(\Delta x, t)$, the fluorescence intensity at a time t after the bleaching pulse, were fitted to the following form

$$F(\Delta x, t) = F(-)\{1-\beta\alpha(t)\exp[-(\Delta x)^2/w^2(t)] - (1-\beta)\alpha_0\exp[-(\Delta x)^2/w_0^2]\} \qquad (1)$$

with

$$\alpha(t) = \alpha_0/(1+t/\tau_\text{D}) \qquad (2)$$

$$w^2(t) = w_0^2(1+t/\tau_\text{D}) \qquad (3)$$

where

$$\tau_\text{D} = w_0^2/4D \qquad (4)$$

is the characteristic time for diffusion; $F(-)$ is the prebleach fluoresence intensity; α_0 is a constant ($0<\alpha_0<1$) which characterises the extent of bleaching; β is the fractional recovery at infinite time; and w_0 is the $1/e$ radius of the assumed gaussian profile of the fluoresence scan at time zero. From each set of data, we computed values of β, τ_D, w_0, and hence D.

Separate control experiments were carried out to check for the possible occurrence of dye-sensitised protein aggregation induced by intense laser irradiation. After the same irradiation doses that produced extensive protein cross-linking in red cell ghosts labelled with fluorescein–concanavalin A (ref. 23), we observed no evidence of protein aggregates on SDS-polyacrylamide gels of labelled vesicles containing 50% protein.

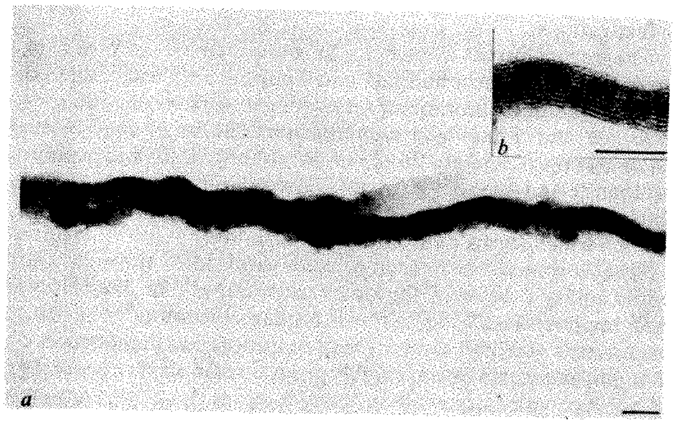

Fig. 3 *a*, *b*, Thin section of multibilayers prepared from vesicles (ratio 1:1.3:1). Multibilayers were prepared by using several techniques cited in Wu *et al.*[12]. A 15-μl portion of the vesicle preparation was applied to a slide resting on a slide warmer set at 45 °C. After drying, another 15 μl was applied. This procedure was followed until 90 μl of vesicle preparation had been deposited on the slide. The slide warmer was then switched off and the slide allowed to reach room temperature. To rehydrate the membrane, a coverslip was sprayed with an aerosol containing 50 mM HEPES, *p*H 7.5, providing a uniform distribution of small droplets. The wetted side of the coverslip was then placed face down on the dried vesicle preparation. A Teflon cover was put over the coverslip and the slide placed between two aluminium blocks. A 25 lb lead brick was then placed on the top block for 20 s. Following pressing, the slide was removed and the coverslip edges sealed with melted paraffin. Samples to be prepared for thin section were sealed with paraffin on only two sides of the coverslip. The multibilayers were fixed in 2% glutaraldehyde and 0.1 M sodium cacodylate, *p*H 7.35, by placing pieces of filter paper over the sample and soaking the paper in fixative. The preparations were left overnight at 4 °C after changing the filter paper and then applying buffer. Post-fixation (1 h, 24 °C, 2×) was carried out with 2% OsO_4 using the filter paper technique of application. After a buffer rinse with filter paper the samples were dehydrated in series ethanols to 100%. During dehydration the coverslip floated off with the multibilayers remaining attached to it. The preparations were then infiltrated with three changes of Epon 812 (multibilayer side), embedded in Epon 812 and hardened at 60 °C for 2 days. Various areas were selected and then re-embedded to allow sectioning normal to the membrane surface. Thin sections (silver) were stained on the grid with 2% uranyl acetate followed by Reynold's lead citrate, 5 min each. Scale bar, 100 nm.

Diffusion of lipopolysaccharide, phospholipid and matrix protein

All diffusion measurements were carried out using a double label: *N*-4-nitrobenzo-2-oxa-1, 3-diazole phosphatidylethanolamine (NBD-PE) and rhodamine-labelled lipopolysaccharide or matrix protein. In each instance, recovery of NBD fluorescence was measured in tandem with measurement of rhodamine recovery on the same patch of reconstituted membrane. All measurements can therefore be related to a phospholipid mobility allowing accurate comparisons. Figure 4*a* shows a set of fluorescence recovery curves for rhodamine-labelled lipopolysaccharide in a multilayer membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The fluorescence was monitored at three equally spaced positions with a 3.0 μm separation. Note that the two curves measured at 3.0 μm on either side of centre are identical, consistent with an isotropic diffusive transport mechanism. The solid lines are theoretical curves calculated according to equations (1)–(4), corresponding to $\beta = 0.80$, $\tau_D = 153$ s, $w_0^2 = 10.8\ \mu\text{m}^2$ and $D = 1.76\times10^{-10}\ \text{cm}^2\ \text{s}^{-1}$. Figure 4*b* shows fluorescence recovery curves for NBD-PE and rhodamine-labelled lipopolysaccharide measured on a single patch of membrane, of the same composition as described above. For simplicity, only the fluorescence measured coincident with the bleaching positions is shown. For comparison, the fractional recoveries

$$f(t) = \frac{F(0, t) - F(0, 0)}{F(0, \infty) - F(0, 0)} \qquad (5)$$

are plotted on a normalised time axis, t/w_0^2. These data correspond to a value of $D_\text{LPS}/D_\text{PL} = 0.14$ (D_LPS and D_PL refer to the diffusion coefficients of lipopolysaccharide and phospholipid, respectively).

Figure 5 is a compilation of our results for NBD-PE and rhodaminated lipopolysaccharide. With the exception of the open points (see below), the data were derived from membrane preparations with a ratio of phospholipid to lipopolysaccharide of 1:1. At 24 °C, the mobilities of lipopolysaccharide and phospholipid are essentially the same in the absence of protein ($D_\text{LPS} \simeq 1\times10^{-9}\ \text{cm}^2\ \text{s}^{-1}$; $D_\text{PL} \simeq 1.5\times10^{-9}\ \text{cm}^2\ \text{s}^{-1}$) (Fig. 5*a*). The mobility of phospholipid in this system is about an order of magnitude slower than reported for model systems containing a single phospholipid species[11,12]. Decreased phospholipid mobilities have already been noted in mixed lipid systems[1,24]. The rate of phospholipid diffusion is practically independent of protein concentration. However, D_LPS decreases by a factor of 10 between 0 and 60% protein content.

The open points in Fig. 5 represent data from membranes with a phospholipid to lipopolysaccharide ratio of 12:1. These values are in line with the others, suggesting that diffusion rates are primarily determined by the total protein concentration and not the individual ratios of phospholipid or lipopolysaccharide to protein. Note also that the diffusion coefficient for lipopolysaccharide is significantly greater at 37 °C than at 25 °C, whereas that for the phospholipid remains practically the same; $D_{\mathrm{LPS}\ 37^{\circ}\mathrm{C}}/D_{\mathrm{PL}\ 25^{\circ}\mathrm{C}} \sim 2$, whereas $D_{\mathrm{PL}\ 37^{\circ}\mathrm{C}}/D_{\mathrm{PL}\ 25^{\circ}\mathrm{C}} \sim 1$ for 40% protein. Matrix protein at concentrations of 40% or 60% demonstrated extremely low diffusion coefficients at room temperature ($\leq 10^{-12}$ cm^2 s^{-1}).

In all our measurements, the phospholipid recovery was ~100% whereas that for lipopolysaccharide was ≥75%. In each case, recovery was monophasic. Thus, there is no evidence of an immoblised 'boundary' lipid; on a time scale of seconds—the time required for diffusion over micrometre distances—all the lipid is exchangeable.

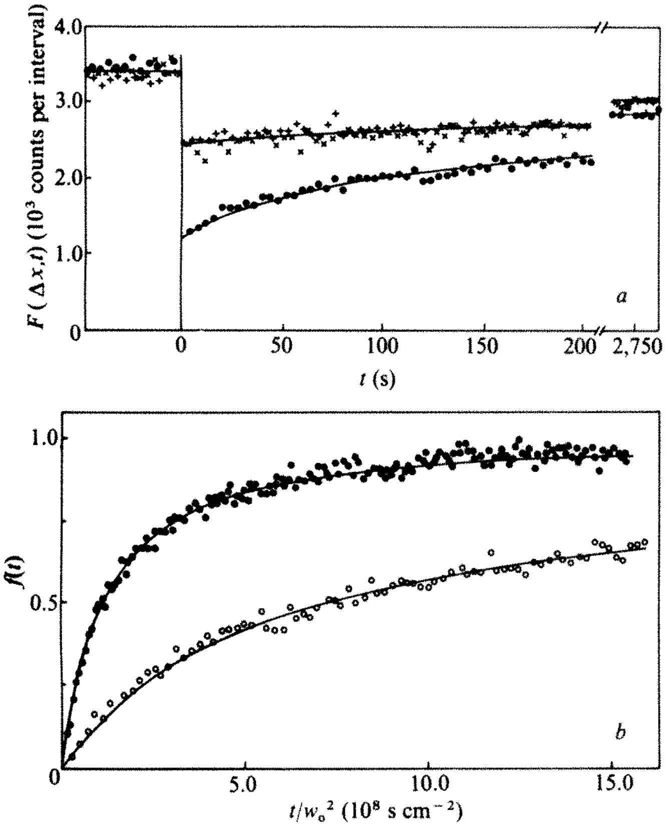

Fig. 4 *a*, Fluorescence recovery curves for rhodamine-labelled lipopolysaccharide in a multibilayer membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The fluorescence was monitored coincident with the bleaching pulse, that is, $\Delta x = 0$ (●), and at equal distances on either side of centre, $\Delta x = \pm 3.0$ μm (+, ×). The solid curves are theoretical values corresponding to $\beta = 0.80$, $\tau_D = 153$ s, $w_0^2 = 10.8$ μm^2, and thus, $D = 1.76 \times 10^{-10}$ cm^2 s^{-1}. Fluorescent components were derivatised in the following way. Electrodialysed G30 lipopolysaccharide (5 mg) was dispersed in 1 ml of 2% $NaHCO_3$, *p*H 8.5. Rhodamine isothiocyanate (5 mg; Kodak) was added and the reaction allowed to proceed for 1 h at 4 °C. The reaction mixture was then chromatographed on a G-25 Sephadex column (1.5 × 25 cm) eluted with water. The rhodaminated lipopolysaccharide was eluted in the void volume, lyophilised, and then resuspended in 1 ml water. The suspension was washed three times with acetone. The lipopolysaccharide precipitated was judged pure by paper chromatography. NBD-PE (Avanti) demonstrated one spot on chromatography. Rhodamine modification of matrix protein involved the addition of 1 mg rhodamine isothiocyanate to a solution of matrix protein (2 mg ml^{-1}) in 2% SDS and 10 mM HEPES, *p*H 7.5. The reaction was allowed to proceed overnight at 4 °C. The protein was then precipitated five times in cold 90% acetone. The residue was lyophilised and then solubilised in 10 mM HEPES, *p*H 7.5. Amino acid analysis indicated that one amino group was modified per protein molecule. In addition, circular dichroism showed that the solubilised protein and rhodaminated protein maintained their predominantly β-sheet conformation (data not shown). Fluorescently labelled analogues of each component were incorporated at molar ratios between 1:100 and 1:1,000 to the unlabelled species. *b*, Fractional recoveries for NBD-PE and rhodamine-labelled lipopolysaccharide measured on the same area of a membrane 1:1.3:1 by weight phospholipid/protein/lipopolysaccharide. The theoretical curves correspond to a ratio of $D_{\mathrm{LPS}}/D_{\mathrm{PL}} = 0.14$.

Biomembranes as polymer networks for diffusion

The lateral mobility of cell membrane components is usually interpreted in terms of a fluid mosaic model[25]. The membrane is pictured as a two-dimensional viscous solution with diffusion coefficients controlled by the membrane viscosity. The theory for diffusion in a two-dimensional fluid was put on a rigorous basis by Saffman and Delbrück[26]. They showed that to a first approximation diffusion coefficients would be inversely proportional to the viscosity of the membrane and the vertical length of the diffusing molecule within the membrane. The theory demonstrated only a weak dependence on the cross-sectional area of the diffusing entity. In the context of the fluid mosaic model one would thus predict the following: (1) molecules as similar in lateral dimension as phospholipid (0.54 nm^2 cross-sectional area) and lipopolysaccharide (2.50 nm^2 cross-sectional area per monomer, molecular weight 3,600)[27] should diffuse at very nearly the same rate, and (2) differences in membrane composition or temperature should change diffusion coefficients by altering membrane viscosity, and hence should affect all coefficients proportionately. These predictions are obviously at odds with our experimental results for multibilayers containing protein.

The data presented in Fig. 5 show that as the concentration of matrix protein increases in the membrane, the mobility of lipopolysaccharide is greatly reduced whereas that for phospholipid remains essentially constant. Moreover, as the temperature increases from 24 °C to 37 °C the diffusion constant of lipopolysaccharide increases significantly whereas that for phospholipid is unchanged.

Our results can be explained in a general way by reintroducing the idea that for some purposes, a biomembrane behaves like a polymeric network. The concept of biomembranes as polymers was first introduced by Lieb and Stein[20,21] to explain transverse diffusion (non-mediated transport) of non-electrolytes across erythrocyte membranes. These studies were later extended to other membranes and demonstrated that, as with polymeric networks, (1) diffusion is a sensitive function of size, (2) diffusion is correlated with the short dimension of the diffusing particle, (3) as the temperature increases, the diffusion rates increase correspondingly more for larger particles than for smaller ones, (4) adding low molecular weight 'plasticisers' (for example, anaesthetics) increases the overall diffusion rates. The results strongly supported the hypothesis that transmembrane diffusion is more accurately described by comparison with non-porous networks of hydrophobic polymers than simple viscous liquids. We propose that the same analogy can be made for translational diffusion of membrane components within the plane of the membrane.

A simple physical model can help provide an understanding of the proposed polymer characteristics. The random thermal motion of polymer chains can be thought of as forming free volumes or 'holes' into which the diffusing molecules can move. The rate of diffusion would then be controlled by the rate of appearance of holes and the probability distribution of hole sizes. Net motion will occur only when a hole is created that exceeds a certain critical size approximately equal to the size of the diffusing molecule. This model thus predicts a sharp size-dependent rate of diffusion. Anything that decreases the average hole size will preferentially slow down the diffusion rate of larger diffusing molecules.

Specific interactions between matrix protein and lipopolysaccharide[14,16,18] could also lead to differential diffusion changes. Such an association mechanism by itself, however, cannot account for the magnitude of the effect observed at low and intermediate protein concentrations. Theoretical values of D_{LPS} were calculated for a model in which specific binding occurs. It was assumed that bound lipopolysaccharide, which would be essentially immobile, is in rapid exchange with free lipopolysaccharide, consistent with our observations of a monophasic recovery. D for free lipopolysaccharide was

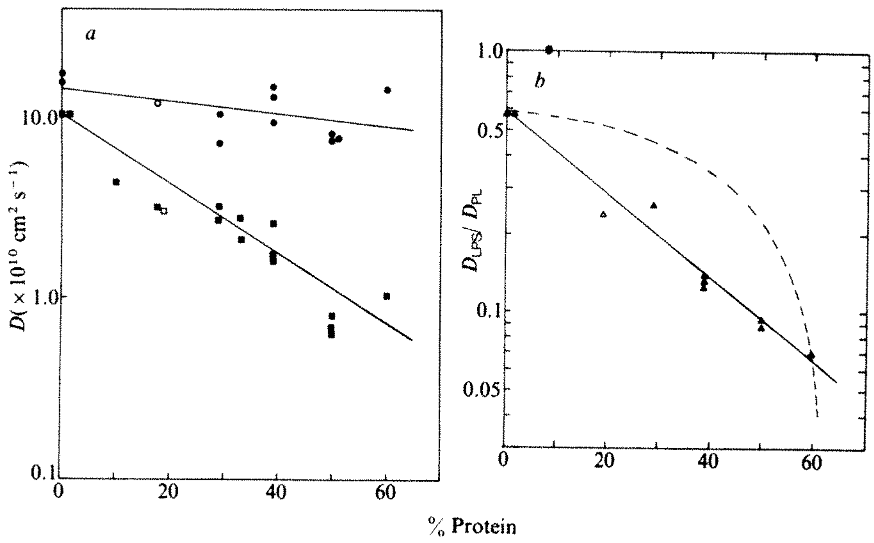

Fig. 5 *a*, Diffusion coefficients of NBD-PE (●, ○), and rhodamine-labelled lipopolysaccharide (■, □) as functions of the percent protein in multibilayer membranes. *b*, Ratios of D_{LPS}/D_{PL}. All ratios were determined from pairs of consecutive measurements carried out on the same patch of double-labelled membrane. The dashed line is a theoretical plot the significance of which is described in the text. The open symbols indicate a preparation with a 12 : 1 (w/w) ratio of phospholipid/lipopolysaccharide. All others correspond to a 1 : 1 ratio.

assumed to vary with protein concentration in a form identical to that found for phospholipids. The dashed curve in Fig. 5*b* corresponds to a stoichiometric binding, with an infinite association constant, of three lipopolysaccharide molecules to a matrix protein monomer[18] in membranes with a phospholipid/lipopolysaccharide ratio of 1 : 1 by weight. Values calculated for lower association constants would deviate from the experimental curve even more significantly. In addition, these calculations predict that for a given protein concentration, D_{LPS} should decrease as the ratio of lipopolysaccharide to protein is decreased. In fact, however, at a protein concentration of 20%, D_{LPS} for a sample with a lipopolysaccharide to protein molar ratio of 3 (open symbols) was essentially indistinguishable from that of samples with a lipopolysaccharide to protein molar ratio of 20.

In the context of the fluid mosaic model, various attempts have been made to relate lateral diffusion to rotational mobility interpreted as membrane microviscosity[1,28]. In our view of membranes as polymeric networks, there need be no simple relationship between lateral and rotational diffusion. Molecules free to rotate rapidly in limited regions might not be able to move laterally over large distances; and changes in membrane composition that moderatly affect rotation rates could have profound effects on the lateral diffusion[28].

The concepts as outlined are particularly useful in relating our findings to the outer membrane of *E. coli*. Our multilayer results indicate that lipopolysaccharide and phospholipids are relatively mobile. However, other evidence suggests that the outer membrane is rigid, with largely immobile components[29,30]. This difference can again be explained in terms of polymer domains. Underlying the outer membrane is a highly cross-linked peptidoglycan structure that is covalently anchored to the outer membrane[31]. The peptidoglycan could thus control the diffusion of outer membrane components. Depending on the degree of cross-linking or attachment of proteins, it is conceivable that some areas exist in the outer membrane that allow greater degrees of mobility than other areas. This might have particular relevence to growth zones in the membrane and phage infectivity.

Schlessinger *et al.*[32] reported diffusion characteristics in cells in which the state of aggregation of one receptor affects the diffusion of another (anchorage modulation hypothesis). These results can be explained by changes in the polymeric nature of the membrane mediated by cytoskeleton components (microfilaments, microtubules).

Investigations of lateral mobilities of proteins in neonatal erythrocytes by Tokuyasu *et al.*[33] have shown membrane domains free of an underlying spectrin network. Lateral mobility in these domains was demonstrated to be significantly greater than in the spectrin-containing areas.

Because our data and most other mobility-related observations can successfully be interpreted using a polymer network approach, we believe that such an analysis might provide a general guide to membrane events. In addition, it would be able to reconcile the dual aspect of membranes—their viscoelastic properties, such as hyperelasticity and shear, and their fluid dynamics. The fluid mosaic model in our interpretation would be a special case of the polymer membrane in which the degree of polymerisation approaches zero.

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The role of gene deletion in the immunoglobulin heavy chain switch

T. H. Rabbitts, A. Forster, W. Dunnick & D. L. Bentley
MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

We have examined the arrangement of the genes for the mouse heavy chain subclasses γ_{2b}, γ_{2a} and γ_1 by the Southern hybridisation procedure. Evidence has been found for rearrangement involving the γ_{2b} and γ_{2a} C_H genes in the DNA of cells making IgG2b and IgG2a respectively. The DNA of an IgG2b-secreting line lacks detectable $C\gamma_1$ gene, whilst the DNA of an IgG2a-secreting line lacks detectable $C\gamma_1$ and $C\gamma_{2b}$ genes. The DNA of a cell line secreting IgA lacks detectable $C\gamma$ genes. These observations implicate deletion in the mechanism of the H-chain switch and allow a preliminary ordering of some of the C_H genes in the mouse genome.

IMMUNOGLOBULIN polypeptides comprise two heavy (H) and two light (L) chains, each in turn consisting of an amino-terminal variable (V) region and a carboxy-terminal constant (C) region. The H-chain constant regions are sub-divided into five subclasses (μ, δ, γ, ε, α) each of which associates with a V-gene at a certain stage of B-cell differentiation. In 1965, the two genes–one polypeptide hypothesis[1] suggested that the V and C regions were separately encoded and that an integration event brought these two segments together. Extensive data now exist on the events involved in the differentiation of the L-chain genes in mouse. For example, it is now known that kappa (κ) chain genes have separately encoded V and C segments[2-4] which are rearranged in the formation of the active κ gene[2,3,5]. The $C\kappa$ gene, in germ-line DNA, is separated by intervening sequences from a set of segments called J-segments[2-5]. Integration involves fusion of a $V\kappa$ segment with one of the J segments and the intervening sequence, being retained between J and C (refs 2–7), is removed post-transcriptionally by RNA splicing enzymes[8,9]. In the H-chain gene system the $C\mu$ seems to be the initial expressed H-chain class[10,11]. However, H-chain gene expression involves the H-chain switch that is manifested by the predominance of IgM (μ-chain class) in the primary response and by the predominance of IgG (γ-chain class) in the secondary response[11]. Also the terminal stage of this pathway can be, in some cases, a switch to IgA (α-chain class) production[11]. At the DNA level this switch would seem to involve a V_H gene first being expressed with one C_H gene (such as $C\mu$), then being 'switched' from this C_H gene to a different one (such as $C\gamma$) and possibly later being switched to a further different C_H gene (such as $C\alpha$). A critical feature of the switch is the expression of a V-region idiotype with the different C_H classes[12,13]. Several hypotheses have been proposed to account for the switch: principal among these are:

(1) *Sequential insertion model.* An initial event integrates a V_H gene with the C_μ gene and subsequently this V_H gene is re-integrated with a $C\gamma$ gene and finally with $C\alpha$ (ref. 14).
(2) *Multiple insertion model.* The integration event involves integration of copies of a V_H gene with each C_H gene[14].
(3) *Post-transcriptional model.* A single integration event is invoked at the most distal part of the C_H gene's array. The V_H gene plus C_H gene array is transcribed as a unit into a precursor RNA which can yield V_H–C_H combinations by RNA splicing[8,15].
(4) *Deletion model.* Integration occurs between a V_H gene and the $C\mu$ gene with subsequent switching facilitated by deletion of material between the integrated V_H gene and the next C_H gene[16].

Each of these theories is testable since the various C_H genes can be regarded as markers for H-chain gene arrangements and rearrangements. Honjo and Kataoka[16] have used cDNA probes (made by reverse-transcribing mRNA) to test these hypotheses by solution hybridisation and they concluded that the H-chain gene switch operates by a deletion mechanism. We have examined the hybridisation of cloned cDNA plasmids, containing γ_{2b} and γ_1 sequences, with genomic DNA made from cells producing different Ig H-chain classes. The results indicate rearrangement involving γ_{2b} and γ_{2a} genes in the DNA of cells producing the respective immunoglobulins and are consistent with the idea that the H-chain switch at least partly involves deletion events.

Cross-hybridisation of mouse γ subclasses

The cloned cDNA probe used in the experiments described here was $p\gamma_{2b}/7$, a cDNA complementary to part of the C_H region of γ_{2b} cloned in the plasmid vector pMB9 (this clone was prepared by Schibler, Marcu and Perry[17,18]). This probe has been used in Southern hybridisation experiments[19] after labelling with ^{32}P by nick-translation[20,21]. Briefly, the hybridisation procedure involves agarose gel electrophoresis of restriction enzyme digested DNA followed by immobilisation of the DNA on cellulose nitrate filters. These filters were hybridised to the ^{32}P-labelled cloned DNA (or cDNA transcribed from purified mRNA with reverse transcriptase), washed and hybridisation bands visualised by autoradiography.

Cross-hybridisation has been reported between the γ subclasses in liquid hybridisation[22]. We have examined the cross-hybridisation between γ_{2a}, γ_{2b} and γ_1 gene sequences using the filter hybridisation method. The $p\gamma_{2b}/7$ clone is cleaved by the restriction enzyme *Hha*I to yield a fragment of about 2.2 kilobases which contains the γ_{2b} cDNA insert[17]. We found by screening a number of different restriction enzymes that the γ_{2b} cDNA insert was internally cleaved by the enzymes *Sac*I, *Pvu*II, *Pst*I, *Kpn*I, *Bst*I (unpublished data). Accordingly $p\gamma_{2b}/7$ DNA was digested with *Hha*I alone or *Hha*I plus one of these enzymes, fractionated on agarose gels and the DNA transferred to cellulose nitrate filters. These filters were hybridised with either nick-translated ^{32}P-labelled $p\gamma_{2b}/7$ DNA (Fig. 1*a*) with cDNA made from purified γ_{2a} H-chain mRNA (Fig. 1*b*) or with cDNA made from purified γ_{2b} H-chain mRNA (Fig. 1*c*). Clearly there is no distinction between the hybridisation of cDNA made from either γ_{2a} or γ_{2b} mRNA and the digests of the $p\gamma_{2b}/7$ clone. Both of these cDNA probes (Fig. 1*b*, *c*) hybridise specifically to the restriction fragments containing γ_{2b} sequence whilst the ^{32}P-$p\gamma_{2b}/7$ clone DNA hybridises in addition to plasmid-derived restriction fragments (Fig. 1*a*). The presence of cleavage sites for the various enzymes within the γ_{2b} insert is

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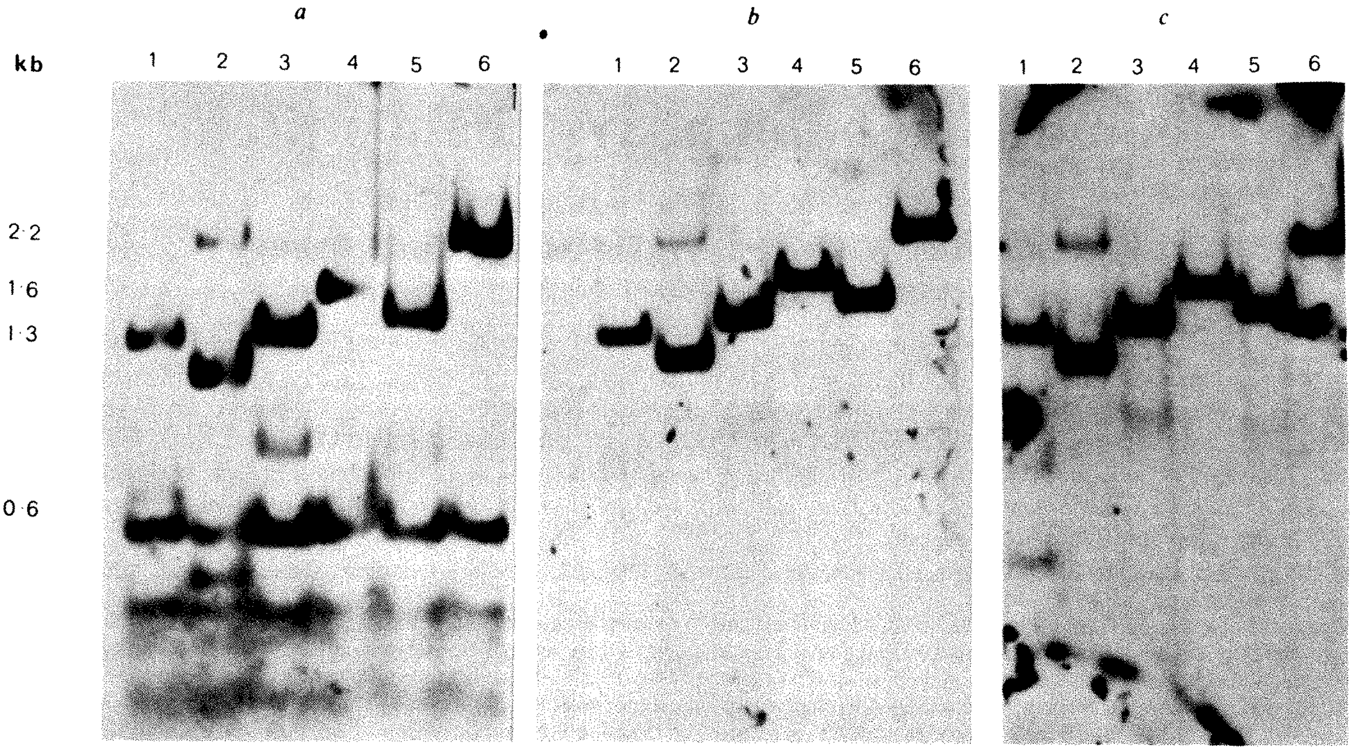

Fig. 1 Cross-hybridisation of pγ_{2b}/7 and cDNA made from cells producing γ_{2a} or γ_{2b} H-chain mRNA. 100 ng pγ_{2b}/7 were digested to completion with *Hha*I and further digested with *Sac*I, *Pvu* II, *Pst*I, *Kpn*I or *Bst*I. The digests were fractionated by electrophoresis on 2% agarose gels, the DNA was denatured and transferred to cellulose nitrate filters[19]. The filters were subsequently hybridised to *a*, nick-translated ^{32}P-labelled pγ_{2b}/7 DNA; *b*, T_{10}-primed ^{32}P-labelled cDNA synthesised on γ_{2a} H-chain mRNA using reverse transcriptase[2]; or *c*, T_{10}-primed ^{32}P-labelled cDNA made on γ_{2b} H-chain mRNA. The hybridisations were carried out for 36 h at 65 °C in 6 × SSC, 0.1% SDS, 50 μg ml^{-1} denatured sheared salmon DNA, 10 μg ml^{-1} poly(A) plus Ficoll, bovine serum albumin and polyvinyl pyrrolidine as prescribed by Denhardt[40] and Jeffreys and Flavell[41]. Post hybridisation washes were carried out at 65 °C, first with 6 × SSC containing the above components and then five washes (30 min in each) with 1 × SSC plus the above additions. A final wash was with 1 × SSC, 0.1% SDS was carried out before overnight autoradiography with prefogged X-ray film at −70 °C (ref. 42). Slot 1, *Hha*I + *Sac*I; slot 2, *Hha*I + *Pvu*II; slot 3, *Hha*I + *Pst*I; slot 4, *Hha*I + *Kpn*I; slot 5, *Hha*I + *Bst*I; slot 6, *Hha*I alone. Molecular weights are given in kilobases (kb).

manifested by reduction in the size of *Hha*I fragments after redigestion with the appropriate enzyme. Evidently, therefore, γ_{2a} and γ_{2b} sequences cross-hybridise with more or less equal efficiency in the hybridisation conditions used. A similar approach has been used to demonstrate the cross-hybridisation of γ_1 and γ_{2b} sequences (data not shown).

Hybridisation analysis of the genes for the γ subclasses in various myeloma cell lines

The various cell lines or tumours used in this study are listed in Table 1 and each cell line was monitored for the production of the respective type of immunoglobulin (by *in vitro* incorporation of ^{14}C-labelled amino acids). The strategy of our experiments has been to use the genomic DNA of these cell lines to hybridise (using the Southern filter hybridisation procedure) with probes for γ_2 and γ_1 genes using hybridisation conditions established in the previous section, where γ_2 and γ_1 genes cross-react to varying degrees. Figure 2 shows the result of a Southern hybridisation between the cloned γ_{2b} DNA and *Eco*RI-digested DNA of various types. The kidney DNA (slot 4) shows two major hybridisation components of about 20 and 7 kilobases respectively. Kataoka *et al.*[23] have recently isolated a genomic DNA clone (from *Eco*RI-digested mouse DNA) which was shown to contain the γ_{2b} C_H-gene by partial nucleotide sequencing. This cloned DNA has a size of around 7 kilobases and allows an unequivocal assignment of the 7-kilobase component as containing the γ_{2b} gene. Interestingly, we find that DNA from cells synthesising γ_{2a} showed only the large hybridising component (slot 2). Since the 20-kilobase fragment is the only hybridisation component observed in this DNA, it can be assigned to the fragment containing the γ_{2a} C_H-gene and the similar component of the kidney DNA will therefore represent the γ_{2a} gene. The hybridisation of γ_{2b} DNA to *Eco*RI-digested DNA of IgG2b-producing cells (Fig. 2, slot 3) and IgG1-producing cells (slot 1) show essentially the same pattern as kidney DNA, except that the IgG1 DNA exhibits a new hybridisation band (about 4.3 kilobases): the origin of this component is not yet known. The data in Fig. 2 show that we cannot detect the γ_{2b} C_H-gene in cells producing IgG2a although we were able to detect this gene and the γ_{2a} C_H-gene in the other DNAs examined.

Two main possibilities may explain our inability to detect the γ_{2b} gene in the IgG2a-producer DNA. Either the gene is absent from this DNA or the H-chain switch involves rearrangements of the γ_{2b} C_H-gene in this DNA in such a way that γ_{2a} and γ_{2b} genes appear in a single fragment. The latter possibility is ruled out by the data in Fig. 3 where we have compared the hybridisation of γ_{2b} cloned DNA to either liver DNA or IgG2a-producer DNA digested with various enzymes. *Bst*I or *Bst*I plus *Eco*RI digests of liver DNA yielded hybridisation bands of 6.7, 5.5 and 5.0 kilobases (Fig. 3), whereas the IgG2a-producer DNA only yielded a 5.5-kilobase fragment. This latter fragment must, therefore, represent the γ_{2a} C_H-gene whilst the 5.0-kilobase hybridisation fragment seen in the liver DNA contains the γ_{2b} C_H-gene[23]. The IgG2a-producer DNA appears not to contain this 5.0-kilobase γ_{2b} fragment. In addition, if the γ_{2b} gene were present in IgG2a-producer cells, *Bst* digestion (which cleaves within the γ_{2b} coding region, see Fig. 1) should yield an additional hybridising fragment due to cleavage within the γ_{2b} sequence. As no such band appears we conclude that the 5.5-kilobase band does not include the γ_{2b} C_H-gene. This conclusion is corroborated by the results of *Kpn* digestion (Fig. 3). *Kpn* or *Kpn* plus *Eco*RI digestion of liver DNA yielded major hybridisation components at 18.5 and 5.1 kilobases whilst the IgG2a-producer DNA yielded only the 18.5-kilobase fragment. By arguments analogous to those used previously, the 18.5-kilobase fragment must represent the γ_{2a} C_H-gene and again, since *Kpn* cleaves within the γ_{2b} insert (Fig. 1), this 18.5-kilobase fragment cannot contain the γ_{2b} gene as an additional hybridising fragment would appear in such a circumstance. The 5.1-kilobase *Kpn* fragment of liver DNA can be assigned to the γ_1 C_H-gene[24] so we conclude that we cannot detect a γ_1 C_H-gene in IgG2a-producer DNA. (This conclusion has been confirmed by the inability of a γ_1 cDNA probe to hybridise this C_H-gene in the IgG2a-producer DNA [unpublished data].) Furthermore, liver DNA yielded a strongly hybridising *Kpn* fragment of 4.2 kilobases which is cleaved by *Eco*RI: the intensity of this hybridisation suggests that the fragment contains the γ_{2b} C_H-gene, and this conclusion has been confirmed by restriction mapping of a genomic γ_{2b} C_H-gene clone[23]. We do not observe any such component in the IgG2a-producer DNA which extends our conclusion that this DNA lacks the γ_{2b} C_H-gene.

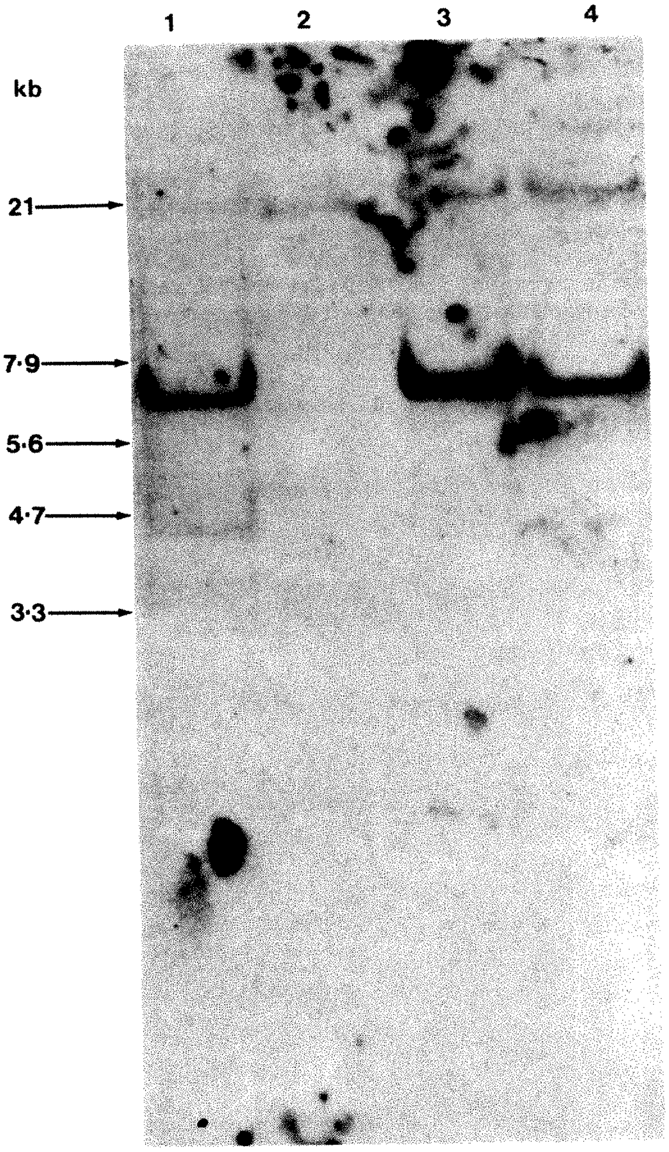

Fig. 2 Pattern of hybridisation of γ_{2b} cDNA to *Eco*RI-digested nuclear DNA from cells synthesising various Cγ subclasses. High molecular weight nuclear DNA[2] from cells producing Ig of the classes γ_1, γ_{2a}, γ_{2b} or DNA of kidney cells were digested to completion with *Eco*RI, fractionated by 0.8% agarose gel electrophoresis and the DNA transferred to cellulose nitrate filters[19]. Hybridisation of the filters was carried out for 48 h with 25 ng ml^{-1} nick-translated pγ_{2b}/7 (specific activity 5×10^7 c.p.m. μg^{-1}) in 6×SSC (hybridisation, post-hybridisation washing and autoradiography was carried out as legend to Fig. 1). This autoradiograph shows hybridisation to DNA of cells making γ_1 (slot 1), γ_{2a} (slot 2), γ_{2b} (slot 3) and DNA of kidney cells (slot 4). Size calibration was carried out by co-electrophoresis of an *Eco*RI digest of λ phage DNA followed by ethidium bromide staining before transfer of DNA to the filters.

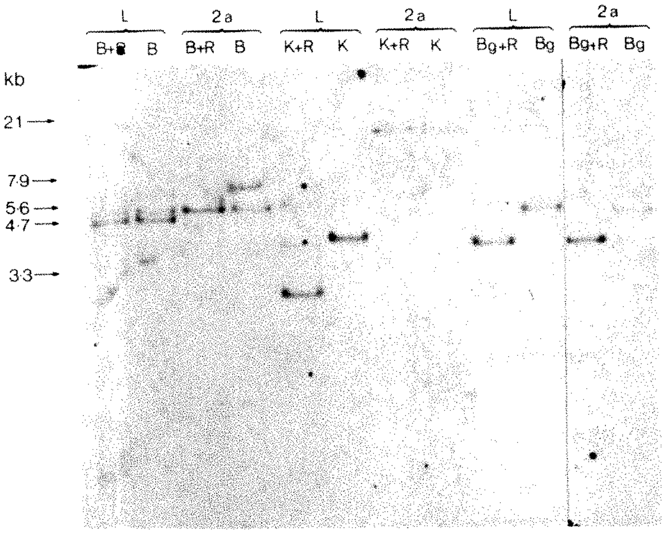

Fig. 3 Hybridisation of γ_{2b} cDNA to liver DNA and DNA of an IgG2a-secretor digested with various restriction enzymes. Liver DNA or DNA prepared from an IgG2a secretor were digested with the restriction enzymes *Bst*I, *Kpn*I or *Bgl*II or mixtures of these enzymes with *Eco*RI. The digests were fractionated and hybridised to ^{32}P-labelled pγ_{2b}/7 as in Fig. 2 legend. B = *Bst*I; B + R = double digest of *Bst*I + *Eco*RI; K = *Kpn*I; K + R = double digest of *Kpn*I + *Eco*RI; Bg = *Bgl*II; Bg + R = double digest of *Bgl*II + *Eco*RI; L = liver DNA; 2a = DNA of IgG2a-secretor.

The comparison of liver DNA and IgG2a DNA in Fig. 3 also includes the results of digestion with *Bgl*II which provides evidence for rearrangement events involving the γ_{2a} C_H-genes. The liver DNA cleaved by *Bgl*II produced two main bands of hybridisation of about 5.2 and 4.9 kilobases. Both of these bands are susceptible to *Eco*RI since double digests produce a new major band at about 3.9 kilobases. The IgG2a cell DNA shows only a single major hybridisation band of about 3.8 kilobases when digested with *Bgl*II plus *Eco*RI: *Bgl*II digestion alone, however, produced a distinctly different pattern of hybridisation compared to liver DNA. Now only the 4.9-kilobase fragment is common between liver DNA and the IgG2a cell DNA whilst new bands of about 8 and 6.3 kilobases occur in the IgG2a cell DNA. One or both of these bands presumably reflects rearrangements resulting from V_H-gene integration with the γ_{2a} C_H-gene within the DNA of cells destined to synthesise IgG2a. In summary, the data in Figs 2 and 3 support the argument that the DNA of the cell line producing IgG2a lacks C_H genes for γ_{2b} and γ_1 and shows that evidence of rearrangement events specifically involving the γ_{2a} C_H-gene can be found in cells making IgG2a.

Further analyses regarding the presence of the γ_1 and γ_2 genes in cells making IgM, IgG1 and IgA are shown in Figs 4 and 5.

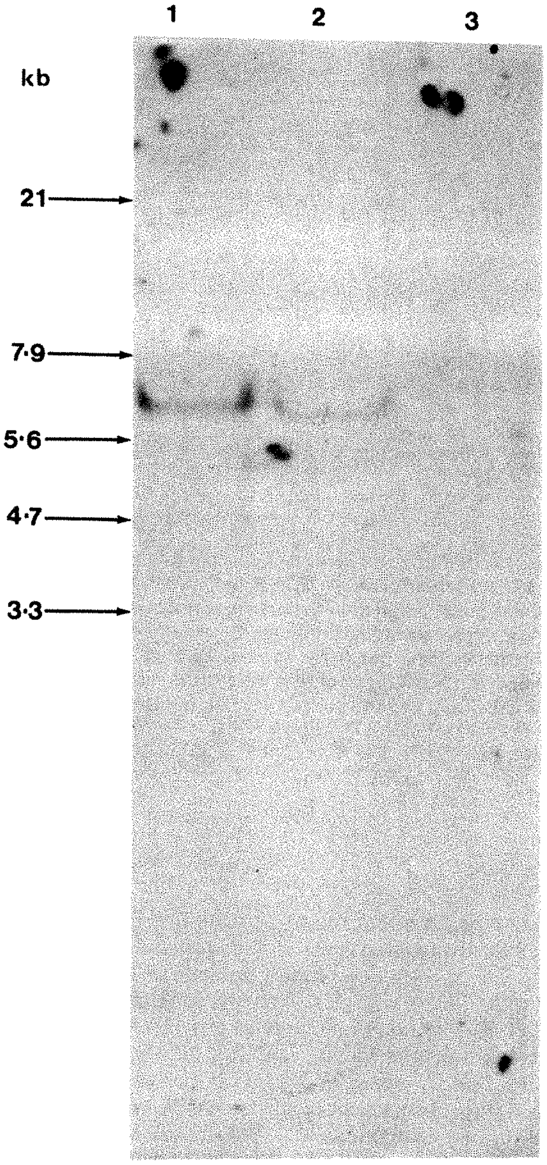

Fig. 4 Hybridisation pattern of γ_{2b} cDNA to DNA from cells making γ_{2b} or α heavy chains. As for Fig. 2, *Eco*RI-digested DNA of liver cells, of cells making IgG2b or IgA was electrophoresed, transferred to filters and these filters hybridised to ^{32}P-labelled pγ_{2b}/7 in 6×SSC followed by washing and autoradiography. Samples loaded were DNA from γ_{2b}-producing cells (slot 1), from liver cells (slot 2) and from α-producing cells (slot 3).

Figure 4 shows the hybridisation patterns of pγ_{2b}/7 with *Eco*RI digests of DNA from the IgG2b secretor (slot 1), liver (slot 2) and an IgA secretor (slot 3). The DNAs of the IgG2b secretor and liver display the characteristic *Eco*RI hybridisation fragments of the γ_{2b} (about 7 kilobases) and the γ_{2a} C_H-genes (about 20 kilobases). The IgA cell DNA (Fig. 4, slot 3), however, failed to hybridise the pγ_{2b}/7 probe, indicating the absence of γ_{2b} and γ_{2a} genes from these cells. Although we do not have a cDNA probe for α-chains, these cells were shown to secrete IgA by *in vitro* incorporation of ^{14}C-labelled amino acids (data not shown). In addition, the same *Eco*RI-digested IgA cell DNA was found to hybridise normally to a κ-L-chain probe. We conclude, therefore, that this IgA-producing cell line does not possess γ_{2a} or γ_{2b} genes.

The presence of both γ_{2a} and γ_{2b} hybridisation components in the DNA of cell lines producing IgM or IgG1 plus clear evidence of rearrangement involving γ_{2b} C_H-genes specifically in the DNA of cells making IgG2b is shown in Fig. 5. In Fig. 5, tracks 6–8 show hybridisation patterns of the pγ_{2b}/7 to various *Eco*RI digests. Track 6 contained *Eco*RI-digested DNA of the IgG2a secretor showing the 20-kilobase Cγ_{2a} hybridisation band. DNA of both the IgM secretor (track 7) and the IgG1 secretor (track 8) shows this hybridisation band. These DNA types also show the γ_{2b} C_H-gene hybridisation band of around 7 kilobases, showing that these cells possess the genes for γ_{2b} and γ_{2a}. Tracks 1–5 show hybridisation of the γ_{2b} probe to *Kpn*-digested DNA. The hybridisation of liver DNA and the IgG2a-producer DNA parallels that shown in Fig. 3 with the γ_{2b} C_H-gene fragment (4.2 kilobases) and γ_{2a} C_H-gene fragment (18.5 kilobases) occurring in liver DNA but only the latter in IgG2a cell DNA. The patterns of hybridisation to *Kpn* digests of IgG1 cell DNA (slot 1) and IgM cell DNA (slot 4) are identical to liver DNA, confirming that the γ_{2a} and γ_{2b} C_H-genes are present in these cells. The hybridisation of pγ_{2b}/7 to *Kpn*-digested DNA of the IgG2b secretor, however, shows a more complex pattern (slot 2). In addition to hybridisation of the γ_{2a} C_H-gene (18.5-kilobase component) and to the γ_{2b} C_H-gene (4.2 kilobases) we observed two new hybridisation bands of about 13 and 6.5 kilobases. These latter bands do not appear in any of the other DNA types studied and must, therefore, originate from rearrangement events which involved integration of the γ_{2b} C_H-gene in precursor cells of these IgG2b producers. These data also suggest that at least two chromosomes carrying γ_{2b} C_H-genes are present in these cells since we observe a mixed pattern

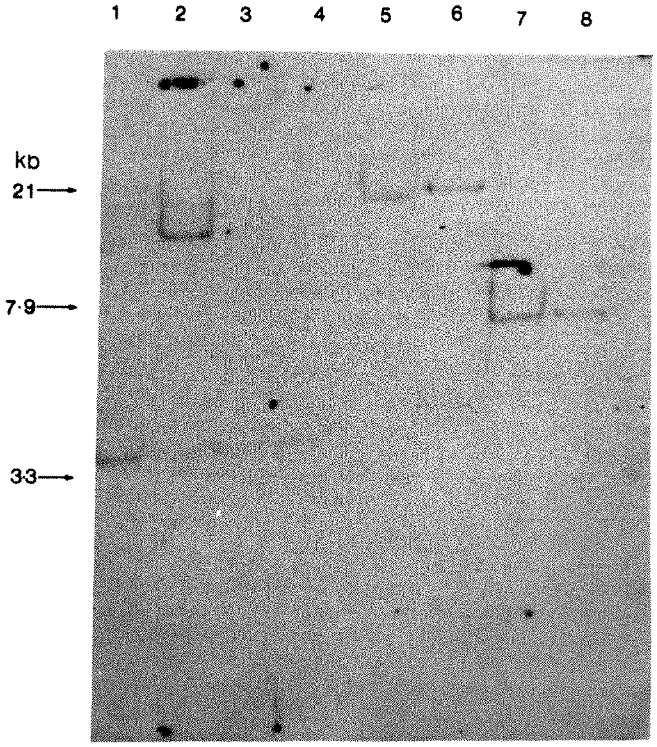

Fig. 5 Hybridisation pattern of γ_{2b} cDNA to *Kpn*I and *Eco*RI digests of various mouse DNA. The DNA of liver cells or cells making heavy chains of the class γ_1, γ_{2b}, γ_{2a} and μ were completely digested with *Kpn*I and DNA of cells making γ_1, μ and γ_{2a} were completely digested with *Eco*RI. The resulting DNA fragments were separated by 0.8% agarose gel electrophoresis, transferred to cellulose nitrate filters and hybridised and washed to ^{32}P-labelled pγ_{2b}/7 DNA in 1×SSC conditions (in which γ_1 and γ_{2b} cross-hybridisation is minimised, unpublished observation). The autoradiograph shows the hybridisation patterns of DNA digested with *Kpn*I (slots 1–5) and *Eco*RI (slots 6–8) and the DNA loadings in the slots are from cells of the type γ_1 (1), γ_{2b} (2), liver (3), μ (4), γ_{2a} (5), γ_{2a} (6), μ (7) and γ_1 (8).

Table 1 Cells used for hybridisation studies

Cell type	Mouse strain	Immunoglobulin produced
McPc 1748	BALB/c	IgM
MOPC 21	BALB/c	IgG1
P1	BALB/c	IgG2a
MPC 11	BALB/c	IgG2b
MOPC 315	BALB/c	IgA
Liver	BALB/c	—
Kidney	BALB/c	—

of the 'rearranged' γ_{2b} components plus a pattern similar to that seen in liver DNA.

The hybridisation conditions used in the type of experiment shown in Fig. 5 minimised the cross-hybridisation of γ_1 and γ_{2b} (unpublished observation). We have also tested for the presence of the γ_1 C_H-gene Kpn fragment in various DNAs in low stringency conditions where γ_1 and γ_2 do cross-hybridise (Fig. 6). Liver DNA and an IgM-producer DNA show (amongst other cross-hybridising genes) the 5.1-kilobase *Kpn* fragment characteristic of the γ_1 C_H-gene[24]. The *Kpn*-digested DNA from the γ_{2b}-producer, however, fails to display this γ_1 C_H-gene component, indicating the absence of this gene from this DNA.

Restriction mapping data implicate gene deletion in the H-chain switch

The data presented in this article show that the DNAs of myeloma cells producing various immunoglobulins can be mapped with respect to their subclass genes using cloned cDNA sequences. This analysis revealed two major features. Evidence of rearrangement events involving C_H-genes for γ_{2a} and γ_{2b} was found in the DNA of cells expressing γ_{2a} or γ_{2b} genes respectively. These rearrangements, analogous to those observed by restriction mapping κ-chain genes[2,3,5] presumably represent alterations of the DNA located around these genes during integration with the V_H gene in the creation of the active transcription unit. The alterations in this respect seem to be independent since we only find evidence of rearrangements specifically involving a C_H gene in the DNA of cells producing the respective class of H-chain, for example, evidence of γ_{2b} rearrangement could only be found in the DNA of cells making IgG2b and not, say, in cells making IgM. However, the alterations seem to interrelate to the extent that when γ_{2a} cell DNA is examined we find evidence of rearrangements affecting γ_{2a} C_H-genes in these cells, but no evidence of the continued existence of γ_{2b} genes in the DNA. Furthermore, the γ_{2a} and γ_{2b} cells lack detectable γ_1 C_H-genes, whilst μ and γ_1-producing cells possess all three subclass genes. IgA-secreting cells fail to show evidence of any of these γ-subclass genes in their DNA. The presence or absence of the detectable genes in the tumours examined are summarised in Table 2 which includes gene assignments based on appropriate synthesis of Ig chains by cell cultures. Honjo and Kataoka have previously reported similar findings concerning the absence of various C_H genes in myeloma cell lines[16]. Previous evidence[10,11] suggests that the switch goes from $C\mu$ to $C\gamma$ to $C\alpha$. Therefore cells making IgM must necessarily contain the $C\mu$ gene, the $C\gamma$ subclasses, plus $C\alpha$, whilst cells making IgA do not necessarily require the previously utilised C_H genes (such as C_γ). In view of the pattern of absence of the C_H genes from the various tumours, therefore, the most compelling conclusion is that the absence of detectable C_H genes is the consequence of deletion operating during the switch of the V_H gene as previously proposed[16]. The switch operates intrachromosomally[25,26] and the putative deletion event must therefore operate in the same way. It is known that there is close linkage between the C_H genes in mouse[27]. In a deletion process, the pattern of absence of C_H genes from cells would allow a preliminary spatial ordering of some C_H genes in the germ-line DNA. This ordering of C_H genes would appear to be $C\mu$, $C\gamma_1$, $C\gamma_{2b}$, $C\gamma_{2a}$, $C\alpha$ (Table 2) and an identical order of C_H genes was obtained by Honjo and Kataoka[16] using solution hybridisation kinetics.

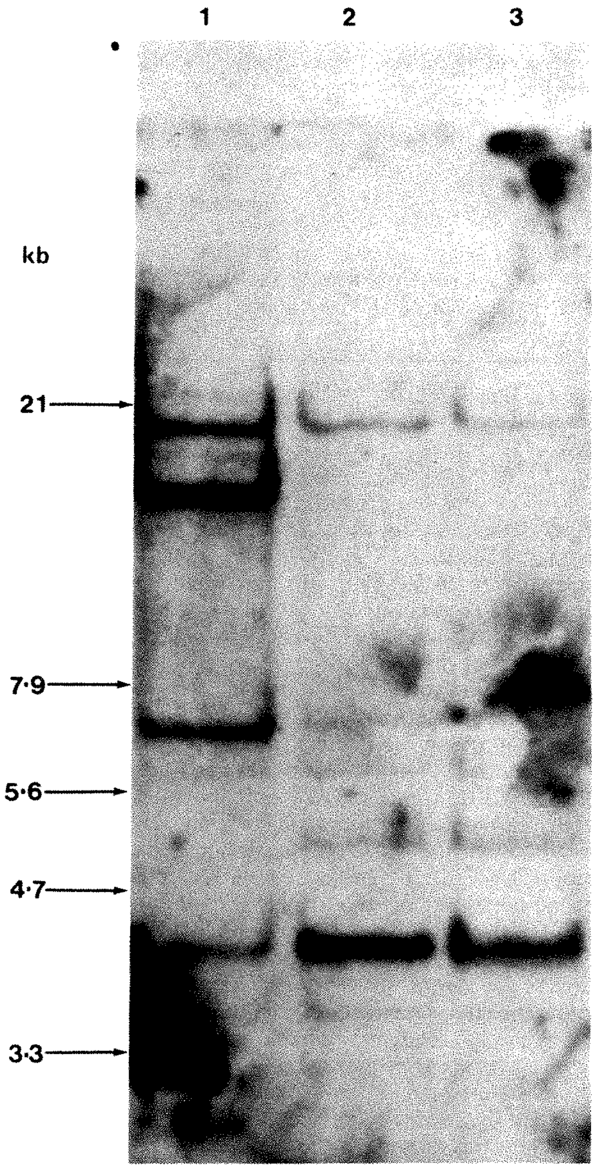

Fig. 6 Hybridisation pattern of *Kpn*I-digested nuclear DNA with γ_{2b} cDNA under low stringency hybridisation conditions. *Kpn*I-digested DNA from γ_{2b}-producing cells, liver cells or μ-producing cells was fractionated and hybridised as in Fig. 4 except that the lower stringency 6 × SSC conditions were used. The slots shown are (1) DNA of γ_{2b} cells, (2) liver DNA, (3) DNA of μ cells.

It is of interest to discuss the possible molecular basis of a deletion process particularly with reference to possible J_H segments. Integration of the V_L with C_L genes involves J segments which are fused with the V segment[6,7,28-30]. It seems likely that similar J segments will occur in the H-chain system[31] and that V_H–J_H fusion will be the initial step of integration of the V_H gene. Therefore we can expect that the selected V_H segment will fuse with one of an array of J_H segments, located close to the $C\mu$ gene. An intervening sequence probably separates the J_H and $C\mu$ of the newly created transcription unit (indeed it has been shown that an intervening sequence does occur between V and

Table 2 Summary of detection of H-chain genes showing probable arrangement of C_H genes in germ-line DNA

		Genes present and arrangement of C_H genes				
		μ	γ_1	γ_{2b}	γ_{2a}	α
C_H gene expressed	μ	√	√	√	√	ND
	γ_1	ND	√	√	√	ND
	γ_{2b}	ND	×	√	√	ND
	γ_{2a}	ND	×	×	√	ND
	α	ND	×	×	×	

√, Relevant gene present in the DNA. ×, Relevant gene was undetected in the DNA. ND, not determined, as no probe was available.

$C\alpha$ in a clone isolated from cells making IgA[32]), and the transcription unit now utilised by the cell will be V J_H ... intervening sequence ... $C\mu$. Assuming that V_H–J_H fusion occurs similarly to the V_L–C_L events, the resulting gene segment will consist of a continuous base sequence of V_H and J_H. This sequence will not contain any non-coding bases between V and J which might act as signals for re-integration of the V_H to a new J_H segment in the switch. The switch must therefore operate differently from V_H integration. Two main possibilities exist. First, it might occur by unequal cross-over between sister chromatids before cell division. The site of recombination might be between similar or identical J_H segments associated with the C_H genes, or sequences occurring within the intervening sequence between the VJ-$C\mu$ transcription unit and between the various C_H genes could be used for the recombination event. The second possibility draws an analogy from the mechanisms proposed for κ-chain integration which invoke the pairing of repeat sequences before $V\kappa$–$J\kappa$ fusion[5,6]. Thus it is possible that the switch of the V gene involves such repeat sequences present both within the intervening sequence of the VJ-$C\mu$ transcription unit and within the sequences separating the various C_H genes. Pairing of such repeat sequences followed by excision of the looped-out material could then facilitate the switching of a V_H gene between C_H genes.

A deletion mechanism for the switch has previously been proposed on the basis of solution hybridisation data by Honjo and Katoaka[16]. These workers deduced that the switch operated on only one of the allelic chromosomes with the non-expressed chromosomes remaining unchanged. Our data suggest the different possibility that the switch occurs on both allelic chromosomes since the experiments show, apparently, the absence of C_H genes from both chromosomes. However, the aneuploid nature of myeloma karyotypes may indicate that the cells used have lost the non-expressed allelic chromosome. We must also consider the possibilities that the deletions which we have observed are merely the manifestation of the aberrant myeloma karyotypes and as such would have no particular bearing on the H-chain switch. However, the observation that rearrangements involving the C_H genes of γ_{2a} and γ_{2b} can be specifically detected in the respective secretor DNA together with the apparent absence of certain C_H genes in the same DNA argues for the authenticity of the conclusion. In addition, experiments testing the rearrangement of some κ-producing myeloma cells[1,2] also indicated possible rearrangement of the non-expressed allelic chromosome, so chromosome loss may not be the full explanation. We are left with the possibility that the V/C integration (both L and H chain) and even the H-chain switch occurs on both allelic chromosomes, and it is possible that such alterations on both chromosomes may explain the presence of extra hybridisation bands in certain digests of γ_{2a} and γ_{2b} DNA.

The involvement of deletion in the H-chain switch may not be the whole answer to the problem, since cells clearly cannot go back to express a gene which has been deleted. Therefore we can ask the question about the situation regarding cells which co-produce μ and δ-H-chains[33,34]. A possible explanation is that these cells contain stable mRNA from one or other of these genes so that continued production of the protein can occur after switch/deletion to the second gene. However, it is still possible that μ–δ co-production occurs by V–C joining in the nuclear RNA precursors. Such a general mechanism was proposed for the H-chain switch[8,15,35] but it has been shown that at least μ and γ C-gene sequences do not co-exist in the nuclear RNA precursors[18]. However, the nuclear RNA precursors of μ/δ-producing cells may contain a precursor of the type $V_HC\mu C_\delta$ from which can be made either mRNA $V_HC\mu$ or $V_HC\delta$ by RNA splicing. This would be a simplification of previous schemes[8,15]. In the context of possible RNA splicing or recombination it is interesting that variant myeloma cells have been detected which show evidence of switching within the γ subclasses[36–39]. These cells may or may not be representations of the normal switch events and DNA cloning of the variant genes should settle this problem.

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LETTERS

Radio emission from radio-quiet quasars

**J. J. Condon*, S. L. O'Dell†, J. J. Puschell‡ &
W. A. Stein‡**

* National Radio Astronomy Observatory, Edgemont Road, Charlottesville, Virginia 22901; and Department of Physics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

† Department of Physics, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

‡ School of Physics and Astronomy, 116 Church Street S.E., University of Minnesota, Minneapolis, Minnesota 55455

In a search for radio emission from bright, optically selected quasars, we detected ~50% of the quasars at 5 GHz. This radio detection rate is so much higher than that for faint quasars (<10%)[1–4], that it requires that the distribution of radio(R)-to-optical(O) luminosity ratios (L_R/L_O) be correlated with optical properties and/or with distance (or cosmic epoch). The values of L_R/L_O in our sample range from ~10^{-2} to <10^{-5} (where radio detection is no longer possible). So broad and smooth a distribution argues against a distinct dichotomy between 'radio-quiet' and 'radio-loud' quasars. The quasars in our sample which remain undetected at radio frequencies must exhibit a spectral-flux distribution rising from the radio to optical, previously considered an 'abnormal' spectrum for a quasar.

Although quasars were originally discovered on the basis of strong radio emission, it now seems that most optically selected quasars are undetectable as radio sources[1–4]. In fact, most optically selected quasars show no clear evidence, at any frequency, for synchrotron radiation. For most quasars, whether radio-quiet or radio-loud, the IR–optical emission is not highly polarised (≲1%) (refs 5, 6), not a simple power law[7], and not highly variable[8]. In contrast, the radio emission from virtually every extragalactic radio source, and the IR–optical continuum radiation in a small subset of quasars, namely, the BL-Lac objects[9,10] and the 'optically-violent-variable' (OVV) quasars[11], seems explicable in terms of the synchrotron process.

There is still no obvious way to predict, on the basis of IR–optical properties, that a given quasar is radio-quiet[7]. Indeed, how quiet is 'quiet'? If there were a distinct dichotomy between 'radio-quiet' and 'radio-loud' quasars[12], the distribution of radio-to-optical luminosity ratios (L_R/L_O) would probably be bimodal. On the other hand, if radio-quiet and radio-loud quasars are merely extrema of the same phenomenon (as is the case for models involving geometric orientation, such as the relativistic-jet model[13–15]), the distribution of (L_R/L_O) for optically selected quasars should be very broad and smooth.

To answer the question, 'How quiet is quiet?', and to examine the distribution of radio-to-optical luminosity ratios (L_R/L_O), we initiated a search for radio emission from bright, optically selected quasars, with the very large array (VLA) of the US National Radio Astronomy Observatory (NRAO). Using the 1.5-m Mt Lemmon (Arizona) telescope of the University of Minnesota (UMinn) and University of California at San Diego (UCSD), we also obtained optical and IR photometry to seek correlations between IR–optical properties and radio emission.

The investigation reported here differs in two respects from most previous searches for radio emission from optically selected quasars[1–4]. First, we considered only the optically brighter, optically selected quasars—typically, at least 10 times brighter than the optical limits used in previous searches. Second, we used the VLA at 4.885 GHz: At this frequency, the VLA is roughly 10 times more sensitive than any other radio telescope. Combining these two factors then, the present investigation probes the radio-to-optical luminosity-ratio (L_R/L_O) distribution down to values ~100 times smaller than those examined in previous searches. In fact, the limiting spectral flux of the 5-GHz search is even slightly smaller than the intended optical limit of the sample selected—namely, 0.6 mJy at 703 THz ($B = 17$ mag). Consequently, to escape radio detection, a quasar from this optically selected sample must have an inverted spectrum—that is, its spectral-flux distribution (perhaps due to the superposition of multiple components) must rise from radio to optical frequencies.

We now briefly describe the samples and the results. A more detailed presentation, including the IR and optical photometry and possible interpretations in terms of physical mechanisms, appears elsewhere[16].

The sample consists of 22 bright, optically selected quasars (Table 1), studied previously[17–21] and contained in the quasar catalogue of Burbidge, Crowne and Smith[22]. During our radio and IR–optical observations (spring 1978 to spring 1979), only 16 of the selected quasars had a B magnitude brighter than 17. While the sample is not complete, it is, we believe, unbiased with respect to radio emission: quasars are neither included nor excluded because of their radio properties.

We detected 9 of the 22 quasars, including 8 of the 16 with $B \leqslant 17$, at a level >0.6 mJy at 4.885 GHz. Most of the radio sources are unresolved on the VLA, and thus are more compact than a few arc seconds. The detection rate is much higher than that of any previous search for radio emission from optically selected quasars; nevertheless, about half the quasars in our bright-quasar sample remain undetected at radio wavelengths, with a standard deviation of 0.15 mJy about a mean of 0.06 mJy. Therefore, as Fig. 1 shows, more than half the quasars have spectral-flux distributions increasing from radio to optical frequencies, that is, negative radio-to-optical spectral indices, $\alpha_R^O < 0$, for $\alpha_R^O \equiv -[\Delta(\ln F_\nu)/\Delta(\ln \nu)]$.

The distribution of α_R^O exhibits no clear separation between 'radio-loud' and 'radio-quiet': it appears smooth from $\alpha_R^O \approx 0.7$ down to $\alpha_R^O \approx 0.0$, at which point the radio emission is no longer detectable for every quasar in the sample. Thus we find no evidence to support the conjecture that 'radio-loud' and 'radio-quiet' quasars belong to two distinctly different populations[12].

Table 1 Effective radio-to-optical spectral indices of bright, optically selected quasars

Coordinate designation	Redshift	Spectral flux at 702 THZ (mJy)	Radio-to-optical spectral index	Refs	
				Sample	Photometry
0007+106	0.089	5.63	+0.36	21	16
0024+224	1.118	0.68	+0.48	19	16
0026+129	0.142	2.33	−0.02	21	16
0054+144	0.171	1.68	<−0.12	20	16
0100+130	2.690	0.58	<−0.03	20	16
0205+024	0.155	2.11	−0.03	19	16
0847+190	0.568	0.56	<−0.03	20	16
0848+163	1.932	0.8	<−0.06	20	22
0906+484	0.118	0.75	<−0.05	21	16
1001+054	0.161	0.97	<−0.07	21	16
1011+250	1.631	0.90	+0.55	17	16
1246+377	1.241	0.47	<−0.01	18	8
1257+346	1.375	0.54	+0.27	18	16
1304+346	0.184	0.82	<−0.06	18	16
1317+277	1.022	1.39	<−0.10	17	16
1318+290	0.549	0.69	<−0.05	17	16
1321+294	0.960	0.67	<−0.04	17	16
1351+640	0.088	4.41	+0.16	21	7
1517+239	1.901	0.34	<+0.01	20	16
1523+214	1.924	0.21	<+0.05	20	16
1525+227	0.253	0.75	+0.43	20	16
2344+092	0.677	1.09	+0.63	21	16

Elsewhere[16], we consider in detail plausible explanations for the observed large range in radio-to-optical luminosity ratios. If

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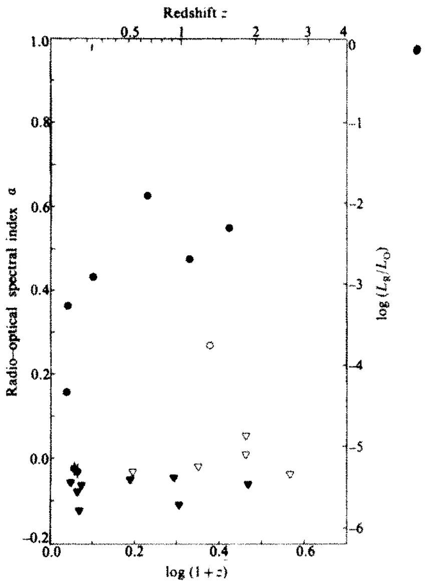

Fig. 1 Distribution of radio-to-optical luminosity ratios (L_R/L_O) for a sample of bright optically selected quasars, plotted against $(1+z)$, with z the redshift. ○, Radio detections; ▽, 2σ upper limits. The filled symbols represent the brighter quasars ($B<$ 17 mag).

the IR–optical emission from quasars is synchrotron (or processed, for example, Compton scattered, synchrotron) radiation, the frequent weakness of synchrotron emission at radio frequencies might result from synchrotron self-absorption or from free–free absorption by intervening material, such as broad-line emitting regions. (This latter explanation is quite reasonable; however, it would be inadequate if truly radio-quiet BL-Lac objects were to be discovered.) If the IR–optical continuum is not synchrotron radiation, there need not be any correlation between radio and optical luminosities. Relativistic-jet models[13,14] owing to differences in orientation, require a rather broad distribution of radio-to-optical spectral indices[15,23]. In this respect, our results are in qualitative agreement with relativistic-jet models; however, agreement in detail requires an hypothesis somewhat more complicated than that considered so far[15] (see below).

Finally, we question why the radio-detection rate for our sample (~50%) is so much higher than that for other searches (~10%)[1–4]. That we consider only optically bright quasars and have utilised the remarkable sensitivity of the VLA partly explains the higher detection rate for our sample. However, even with a 100-fold less sensitive radio search ($F_\nu<60$ mJy, or 100 mJy, say), we would still have detected about 30% (5 out of 16) of the quasars with $B\leq17$ mag.

It seems, therefore, that quasars of bright apparent magnitude exhibit a significantly higher incidence of radio emission than would be expected on the basis of a scaling of detection thresholds. In as much as the optical-selection techniques are unbiased with respect to radio emission, the fraction of quasars with (L_R/L_O) or ($-\alpha_R^O$) above some threshold should be independent of the limiting optical flux or any other optical-selection criterion provided that the ratio (L_R/L_O) is uncorrelated both with optical properties and with distance (and, hence, cosmic epoch). That this fraction does depend on the limiting optical flux, shows that the distribution of (L_R/L_O) must depend on optical properties and/or epoch (or distance). Owing to the small size of the present sample, one cannot readily determine which of these dependences is more important (see Fig. 1); however, the more rapid evolution observed for optically selected quasars, in comparison to that observed for flat-spectrum radio sources[24], suggests that evolution in the distribution of (L_R/L_O) plays some part.

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A new mode of X-ray bursts from MXB1730−335

H. Inoue, K. Koyama, K. Makishima, M. Matsuoka, T. Murakami, M. Oda, Y. Ogawara, T. Ohashi, N. Shibazaki, Y. Tanaka & Y. Tawara

Institute of Space and Aeronautical Science, University of Tokyo, Meguro, Tokyo 153

I. Kondo

Institute for Cosmic Ray Research, University of Tokyo, Tanashi, Tokyo 188

S. Hayakawa, H. Kunieda, F. Makino, K. Masai & F. Nagase

Department of Astrophysics, Faculty of Science, Nagoya University, Chikusa, Nagoya 464

S. Miyamoto, H. Tsunemi & K. Yamashita

Department of Physics, Faculty of Science, Osaka University, Toyonaka, Osaka 560, Japan

We report here on the unusual behaviour of MXB1730−335, the rapid burster[1] as observed by the X-ray astronomy satellite Hakucho between 8 and 22 August 1979[2]. MXB1730−335 is a unique burst source which generates rapidly repetitive bursts lasting between a few and tens of seconds. In the present observations, however, its behaviour was very different to that previously observed, in that the bursts of a trapezoidal profile were dominant. The profile is characterised by a flat top lasting between 30 s and 10 min. A long train of such trapezoidal bursts was seen for the first time and clearly revealed a new mode of the rapid burster.

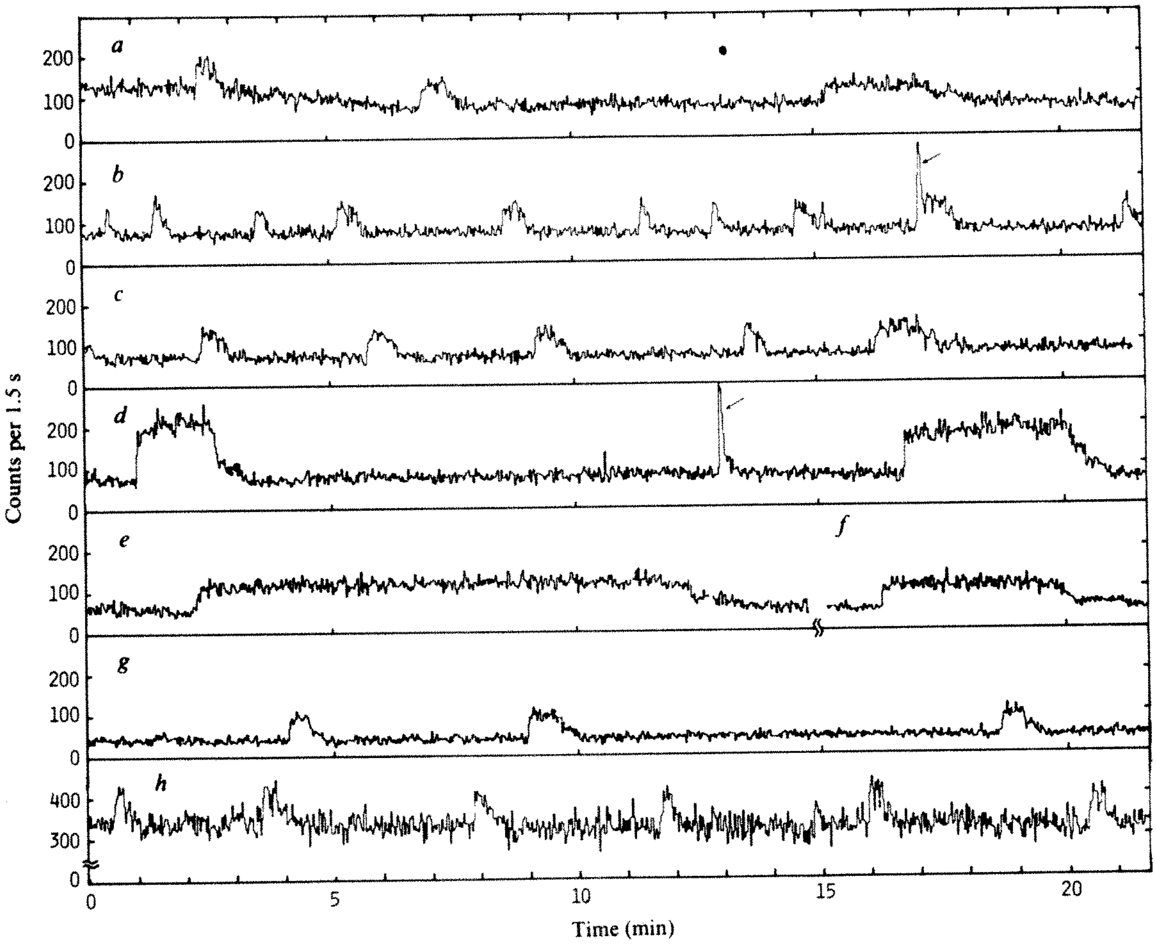

Fig. 1 Segments of the X-ray count-rate record. The graphs *a–g* are from counter FMC 2, and *h* is the sum of the rate from CMC 1 and 2. Since the exposure of the target and the covered energy range (*a*, *b*, 1.5–10 keV; *d–h*, 3–10 keV) are not corrected for, displayed count-rates should not be directly discussed. Two bursts indicated by arrows are from MXB1728 – 337, a nearby burster. Each segment starts at the following times (UT): *a*, 8/8, 1 h 55 min 19.2 s; *b*, 8/8, 3 h 37 min 39.8 s; *c*, 8/8, 5 h 19 min 45.3 s; *d*, 8/8, 19 h 48 min 00 s; *e*, 8/11, 19 h 19 min 54.3 s; *f*, 8/12, 16 h 08 min 24.1 s; *g*, 8/14, 17 h 22 m 58.3 s and *h*, 8/17, 13 h 43 min 39.1 s.

The observation was carried out with an X-ray burst monitor which consists of two sets of rotating modulation collimators: two coarse modulation collimators (CMC 1,2) and a fine modulation collimator (FMC 1) with a field of view of 17.4° and 5.8° (FWHM), respectively. Positions of steady sources and the burst, when it occurs, can be determined with the burst monitor to an accuracy of 0.5° or better. An additional counter (FMC 2) with 5.8° field of view (FWHM) provides non-modulated source signal for comparison. Further details of the instrumentation and the performance of Hakucho are described elsewhere[3].

Since 31 July the burst monitor has been observing a sky region which includes the galactic centre. At 0 h 10 min UT, 8 August, a source in the field of view, whose position was determined with the CMC in a 0.5° error circle including MXB1730 – 335, began to generate a train of bursts (the source was soon confirmed by the FMC 1). The activity of the source was watched through 22 August. The turn-on of the rapid burster was roughly on schedule as expected from the suggested periodicity[4,5] of ~6 months: the previous active period was early March 1979[6].

Figure 1 shows several segments of the X-ray count-rate record. In the early period of the activity starting at 0 h 10 min UT of 8 August, unusual bursts of a trapezoidal profile appeared among bursts of a normal exponential shape (Fig. 1*a*). The data from 3 h 37 min to 4 h 00 min UT of 8 August (Fig. 1*b*) show characteristic features of bursts from the rapid burster such as those observed by SAS 3 (refs 1, 7). After a pause of data acquisition for ~6 h, a long burst train entirely trapezoidal in

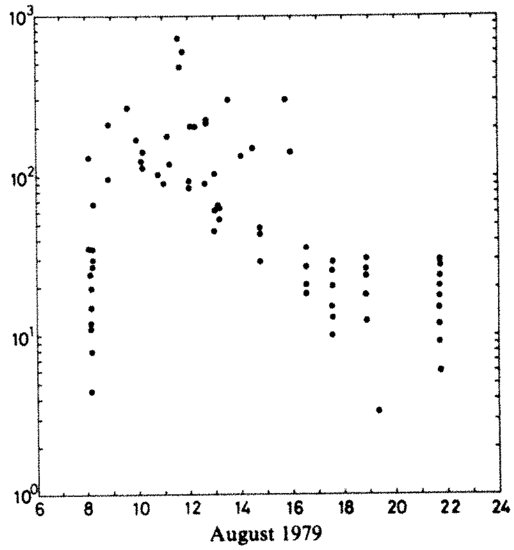

Fig. 2 Plot of the burst duration measured at half maximum of the burst height. Not all the bursts, particularly those of short duration, are plotted. Most bursts of the duration longer than 30 s are of trapezoidal shape. The error of the duration is ~3 s and ~5 s for the period before and after 17 August respectively.

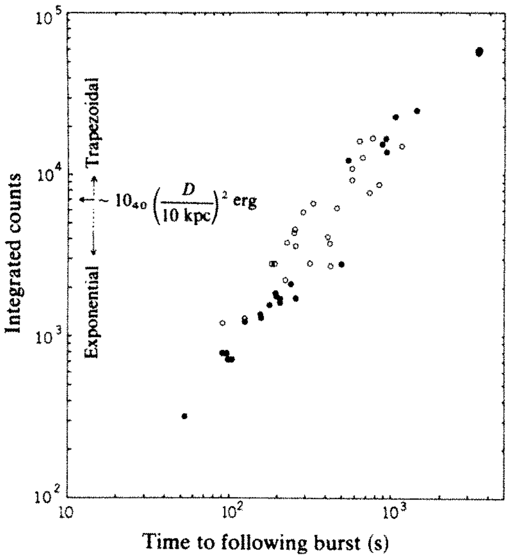

Fig. 3 Integrated counts for a burst over 1.5–10 keV versus the time to the following burst. Correspondence between the total count within an error of ±15% and the total energy is indicated on the ordinate. Note, however, that the energy estimate is tentative. ●, 8.0–11.8 August; ○, 12.5–18.9 August 1979.

form appeared (Fig. 1*d*, *e*). About 90 trapezoidal bursts were observed between 8 and 16 August during a total exposure time of ~35 h.

The predominance of such trapezoidal bursts is new property of the rapid burster[7], although a few such unusual bursts from the rapid burster were noted during the previous turn-on period (ref. 6 and personal communication from W. Lewin).

The burst durations (FWHM) observed for selected satellite orbits are plotted in Fig. 2. The burst duration increased soon after the turn-on of the rapid burster, exceeding 10 min at its maximum. After 12 August trapezoidal bursts of shorter duration predominated (Fig. 1*g*), while the duration scatters. Then, on 16 August bursts of an exponential shape again appeared among trapezoidal bursts and after 17 August, the feature became similar to that of the normal rapid burster (Fig. 1*h*). The activity continued until 22 August when we lost MXB1730–335 from our sight because of the solar direction constraint.

The time profile of the trapezoidal burst is characterised by the following features: a fast rise within ~1 s, a nearly constant plateau and a slow fall to half the maximum in 10–30 s with the association of a shoulder or an after pulse in the decaying phase. These features seem to be independent of the pulse width. The peak luminosity of the trapezoidal bursts is of the order of 10^{38} $(D/10\mathrm{kpc})^2$ erg s^{-1}, D being the distance to the source, but is found to vary by as much as a factor of four. From a simple analysis, the spectral hardness ratio is found to remain constant within statistical uncertainty throughout the flat portion of the trapezoidal burst.

For cases when two or more bursts occurred in an uninterrupted observation window, the integrated counts in each burst (burst size) are plotted against the time interval to the succeeding burst in Fig. 3. While the estimate is tentative, the total energy for most of the trapezoidal bursts $>10^{40}$ $(D/10\mathrm{kpc})^2$ erg. The correlation between the burst size and the interval is similar to that shown by the SAS 3 result (ref. 7 and refs therein); that is, the larger the burst size, the longer is the time until the next burst.

The average slope of the points in Fig. 3 is somewhat steeper than a linear relationship. Whereas, we note episodes in which the burst size versus interval relation is closely linear and hence the time-averaged luminosity is about constant. For example, the filled circles in Fig. 3 indicate two such episodes. The slope is greater than unity because an increased average luminosity tends to favour a larger burst, although the burst size fluctuates. The average luminosity was found to vary by a factor of three over the period of the present observation, during which the burst size spanned two orders of magnitude.

The train of trapezoidal burst is probably a new mode of the Type II burst (ref. 7 and refs therein) which is presumably caused by an instability of the accretion flow onto the neutron star surface. The present data indicate that the trapezoidal bursts predominate when the average luminosity is above a certain value. This implies that a relatively small change in the mean accretion rate corresponds to a drastic change in the burst profile. Instead of increasing the frequency of usual bursts of exponential shape, trapezoidal bursts of various widths are produced with the peak luminosity near the Eddington limit for a 1 $M_\odot$ star. Note, however, that this peak luminosity is also variable. This newly discovered mode of burst undoubtedly imposes a strong constraint on the model of the rapid burster.

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Neutral ^{14}N in the interstellar medium

A. A. Konovalenko & L. G. Sodin

Institute of Radiophysics and Electronics, Kharkov, USSR

The transition frequencies between the hyperfine structure sublevels of atomic nitrogen lie in the decametre waveband. As Shklovsky[1] predicted, these transitions can be observed in the form of narrow spectral lines. The ^{14}N ground state ($^4S_{3/2}$, nucleus spin $I = 1$) is split into three sublevels $F = 5/2$, $3/2$ and $1/2$ with frequencies and transition probabilities

$$\nu = 26.127\ \mathrm{MHz},\quad A = 4.32\times10^{-20}\ \mathrm{s}^{-1}\ \text{for}\ F = 5/2 \rightarrow 3/2$$

and

$$\nu = 15.676\ \mathrm{MHz},\quad A = 1.29\times10^{-20}\ \mathrm{s}^{-1}\ \text{for}\ F = 3/2 \rightarrow 1/2$$

The Einstein coefficients given here have been calculated from the improved values of line intensities[2]. We describe here an attempt made between 1978 and 1979 at the Institute of Radiophysics and Electronics, Academy of Science of the Ukrainian SSR, to detect the ^{14}N absorption line for some point and extended cosmic objects. This effort has been successful for Cassiopeia A.

The observations were carried out using the UTR-2 radio telescope[3] at a frequency of 26.13 ± 20 kHz (collective area of the antenna about 5×10^4 m^2). The emission spectrum was measured with a spectrometer[4] consisting of a receiver with a frequency synthesiser, a digital correlator and a computer controlling the antenna beam and converting the autocorrelation function into the spectrum. Measurements of autocorrelation functions were taken with the beam directed to the source of interest alternatively by the maximum and the first zero (for between 0.5 and 1s). The two values were further subtracted instrumentally. The difference autocorrelation functions were stored and converted into the spectrum. The total storage time of the difference autocorrelation functions was from 1 to 5 h in one observation night. The resolution varied in different observation sessions from 1 to 4 kHz.

The 26.13 MHz absorption line was first detected in the signal of Cas A on 17 June, 1978 after which measurements carried

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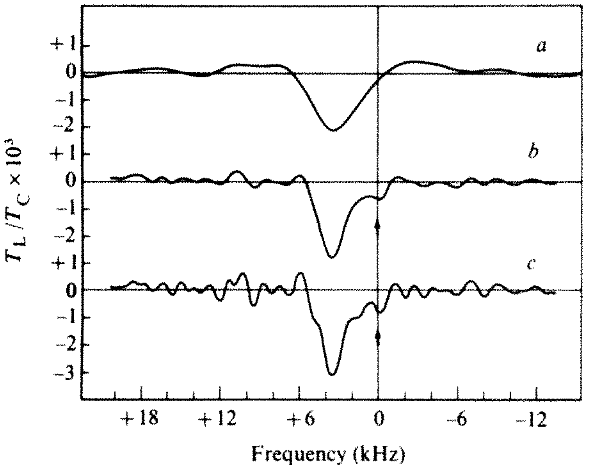

Fig. 1 Absorption spectra in the ^{14}N line $F = 5/2–3/2$ for Cassiopeia A. *a*, Resolution 4 kHz, storage time $T = 26$ h; *b*, resolution 2 kHz, $T = 25$ h; *c*, resolution 1 kHz, $T = 24$ h. The arrow shows the frequency corresponding to the Orion arm.

out over the next year established a coincidence of the observed changes in the mean frequency of the line with the orbital Doppler shift (0.67 kHz with respect to the standard of rest).

Figure 1 shows three spectra from Cas A plotted with different resolutions in the frequency-T_L/T_C scale (where T_L and T_C are temperatures in the line and the continuum). Figure 2 shows the predicted Doppler frequency shift due to the orbital motion of the Earth and measured mean frequencies of the line for different observations. Figure 3 shows the H I absorption line in terms of velocities instead of frequencies[5] and the ^{14}N line reduced to a zero orbital velocity. Strong absorption can be seen in the Perseus arm ($V = -38$ to -48 km s^{-1}) and weak absorption in the Orion arm ($V = -0.8$ km s^{-1}). The measured optical depths at the line minimum are 3×10^{-3} for the Perseus arm and 5×10^{-4} for the Orion arm. The expected absorption line width in the Orion arm[5] is ~0.4 kHz. Hence, the intensity of this part has been underestimated in our measurements.

Thus, the periodic change of frequency coinciding with the orbital Doppler effect and comparing the H I in Cas A spectrum suggests that we have discovered the $F = 5/2 \rightarrow 3/2$ line of ^{14}N.

Measurements for Cygnus A have shown that in its direction $\tau < 2 \times 10^{-4}$; measurements for the Galaxy centre were greatly hampered by interference from broadcasting stations and only allowed us to estimate $\tau < 6 \times 10^{-4}$.

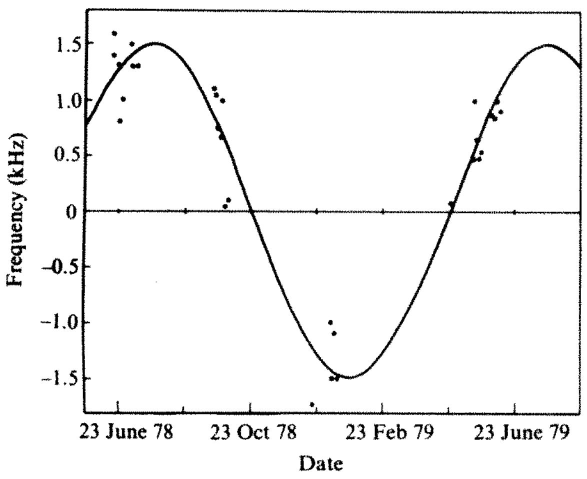

Fig. 2 Change of frequency due to the orbital motion of the Earth. Solid line, calculations; ●, measurements.

To estimate the abundance of nitrogen in the absorbing arms we use the relationship

$$S = \int \tau(\nu)\,d\nu = \tau_{\max}\Delta\nu_{\text{eff}} = \frac{\hbar c^2}{4kT_S}\frac{A}{\nu}\frac{g_F}{\Sigma g_F}n$$

where $\tau(\nu)$ is the line profile; $\hbar$, k, c are Planck's constant, Boltzmann's constant and the velocity of light, respectively; A, and ν are the probability and frequency of the transition; g_F the statistical weight of the sublevel; T_S the spin temperature; and n the density of atoms.

For ^{14}N we have $n_N = 0.7 \times 10^{18} S_N T_{SN}$ (subscript N denotes the values for nitrogen). Our measurements give $S_N \simeq 6$ s^{-1} for the Perseus arm, hence assuming $T_{SN} = 50$ K, we find $n_N = 2.8 \times 10^{20}$ cm^{-2}.

Comparing the abundance of ^{14}N using the H I line, in accordance with Schutter and Verschuur[5], S_H can be estimated as $S_H \simeq 10^6$ s^{-1}, and with $A_H = 2.85 \times 10^{-15}$ s^{-1} and $\nu_H = 1.42 \times 10^9$ Hz we obtain

$$\frac{n_H}{n_N} \simeq 130 \frac{T_{SH}}{T_{SN}}$$

If we assume $T_{SH} = T_{SN}$, the abundance of nitrogen with respect to hydrogen would be more than one order of magnitude higher than the average value in the Galaxy (that is 5×10^{-4}). Other explanations of the unexpectedly strong absorption in the ^{14}N line are also possible.

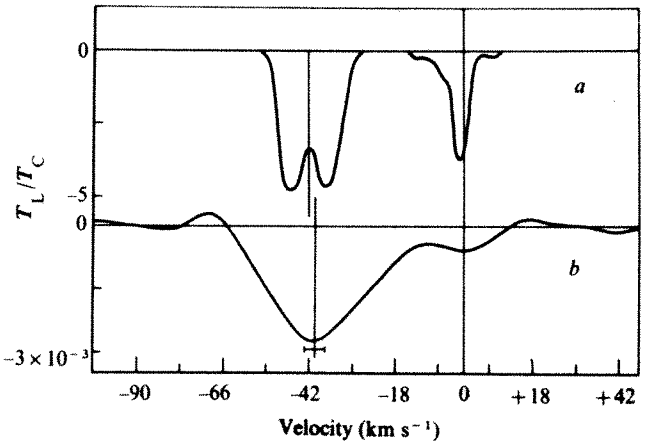

Fig. 3 Velocity spectra in the absorption clouds of the Orion and Perseus arms. *a*, H I line, 1,420 MHz; *b*, ^{14}N line, 26.13 MHz.

(1) The true value of S_H in the gas cloud of the Perseus arm is $S_H \gg 10^6$ s^{-1}. Other measurements[5] allow any value of $\tau_H > 4.7$. In this case the estimate for hydrogen density would need to be increased by more than an order of magnitude and the OH/H I relationship (see ref. 6) revised assuming a higher value for τ_H.

(2) The spin temperature $T_{SN} \ll T_{SH}$: this could be the result of either some specific features in the population of the ^{14}N levels (which can be checked by a theoretical calculation) or nitrogen distribution in the colder parts of the gas cloud.

Further investigations are planned with the aim of discovering the line $F = 3/2 \rightarrow 1/2$ ($\nu = 15.7$ MHz).

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Catalytic combustion in a tube with electrical discharge: preliminary measurements of augmented reaction rates

F. A. Kulacki & S. Boriah

Department of Mechanical Engineering, The Ohio State University, Columbus, Ohio 43210

Since Senftleben[1] first indicated that in the presence of electric fields, heat transfer rates may be increased substantially beyond natural convection values, correlations have been proposed relating electric field and fluid flow parameters to film coefficients of transfer. The 'corona wind', which exists in a non-uniform electric field before spark breakdown, can be used to alter the hydrodynamics of convective diffusion processes such that an augmentation of transport rates in wall boundary layers can be achieved[2–6]. Corona wind augmentation of wall reactions, particularly catalytic combustion processes, has not previously been considered[7,8], but as catalytic combustion is a convective diffusion process, the superposition of the corona wind on the flow field near the catalytic surface may augment overall reaction rates by increased mixing. For very rapid surface reactions, where molecular diffusion inhibits the overall rate and lowers overall rate constants, corona wind augmentation may limit the effects of diffusion and, thereby, increase catalyst effectiveness. Improvements in the overall rate constant, or mass transfer coefficient, would be useful in many areas, including air pollution control, energy conservation, and the design of compact, controlled heat flux catalytic combustors. We report here our test of the catalytic combustion of hydrogen at one atmosphere on platinised alumina in a flow reactor with a uniformly accessible catalyst surface. By this experimental design we may be able to obtain reaction rate data from the kinetically controlled to the diffusion limited regimes of combustion owing to the high activity of hydrogen on metallic platinum. A uniformly accessible catalyst surface also permits analysis of the reaction rate data using either one- or two-dimensional approaches.

The experimental reactor is an annulus of large radius ratio, and the corona discharge is applied from a small diameter (0.053 cm) stainless steel wire suspended under tension along the centreline. The outer cylinder is a 95.5% Al_2O_3 tube of dimensions 4.50 cm o.d. × 3.91 cm i.d. × 58.4 cm long. This tube is coated internally with platinum at an average of 13.3 g m^{-2}. The reactor wall is maintained at a constant temperature to within ±5 °C with electrical heaters, and bulk flow temperatures are held at about one-half the wall value to minimise the possibility of gas phase reaction. The catalyst surface is grounded and a positive d.c. potential (300 V maximum a.c. ripple over the range 6–11 kV) is applied to the central wire. Premixed hydrogen and air enter the reactor in nearly fully developed flow. The combustion of hydrogen is determined by measurements of both inlet and outlet bulk concentrations with a gas chromatograph.

Reaction rate data as a function of average mole per cent of hydrogen, $\bar{y}$, at a constant corona current, I, at a wall temperature, T_w, of 427 °C are presented in Fig. 1. At this wall temperature, the reaction is diffusionally limited in laminar flow[9–10], and the rate data exhibit the expected first-order dependence on $\bar{y}$. Rate data at lower wall temperatures[11] are also first order with respect to $\bar{y}$. From the data of Fig. 1, increases in the reaction rate of the order of 20–25% are observed for moderate levels of corona current. These increases may be interpreted as an augmentation of the mass transfer coefficient owing to the uniformly accessible catalyst surface[12].

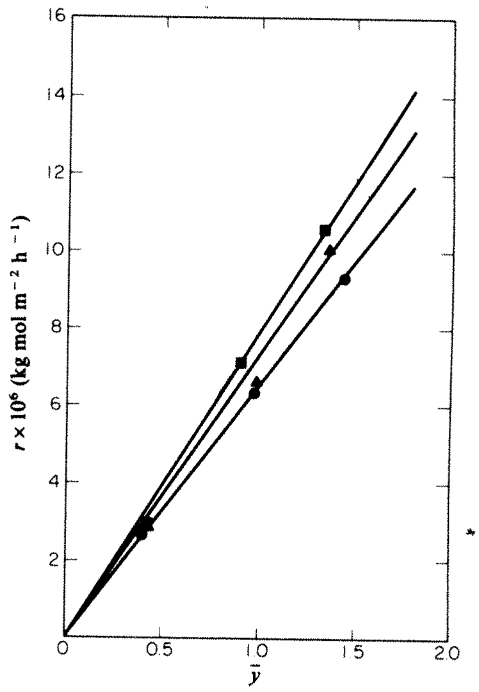

Fig. 1 Reaction rate for combustion of hydrogen on platinised alumina at constant corona current where $T_w = 427$ °C; $T_B = 260$ °C; Re = 1,437. ■, $I = 500$ μA; ▲, $I = 300$ μA; ● = 0.

The combined roles of the Reynolds number and corona current are shown in Fig. 2. Reaction rate data versus corona current for given values of Reynolds number and inlet concentrations of hydrogen, y_i, are presented in Fig. 2*a* in terms of the fractional increase of reaction rate due to the corona discharge. The range of corona current is somewhat larger than shown in Fig. 1 and includes all of the values possible before spark breakdown. For a given value of I, the increase in reaction rate depends on the Reynolds number and y_i in a complex fashion (Fig. 2*b*). At low Reynolds number, the reaction is nearly complete, and $\Delta y/y_i$ is close to unity. Thus, the presence of the corona discharge results in a small increase in reaction rate even though corona wind velocities are of the order of the bulk convective velocity. At larger Reynolds numbers, $\Delta y/y_i$ is smaller, but because the bulk convective velocity is large enough to eliminate essentially the influence of the corona wind, a reasonable augmentation of the rate is obtained. At still larger Reynolds numbers, for example, $Re = 2{,}617$ in Fig. 2*a*, greater fractional increases in reaction rate are obtained. At this Reynolds number, the flow is probably turbulent, but apparently not of large enough velocity to wipe out the influence of corona discharge on the hydrodynamics at the catalytic wall. Limitations of the experimental reactor prohibited us from obtaining reaction rate data at larger Reynolds numbers at this wall temperature. However, one can expect that at larger Reynolds numbers, the axial convective transport would completely dominate the mass transfer problem and that the corona discharge would result in no augmentation of the reaction rate. A similar transition to turbulent flow behaviour has been observed in the heat transfer problem for both tube flows and external boundary layers[13–14]. Note that a cross-plot of data similar to that of Fig. 1 would yield different fractional augmentation values, but the same conclusions would be reached on the role of the Reynolds number.

When the reaction rate data are plotted as a function of Reynolds number at a constant corona current, the fractional augmentation of reaction rate exhibits the highly non-linear behaviour shown in Fig. 2*b*. Here, increases in Reynolds number past a certain value (depending roughly on wall temperature and corona current) result in a rapid decrease in $(r_e - r_c)/r_0$. This is

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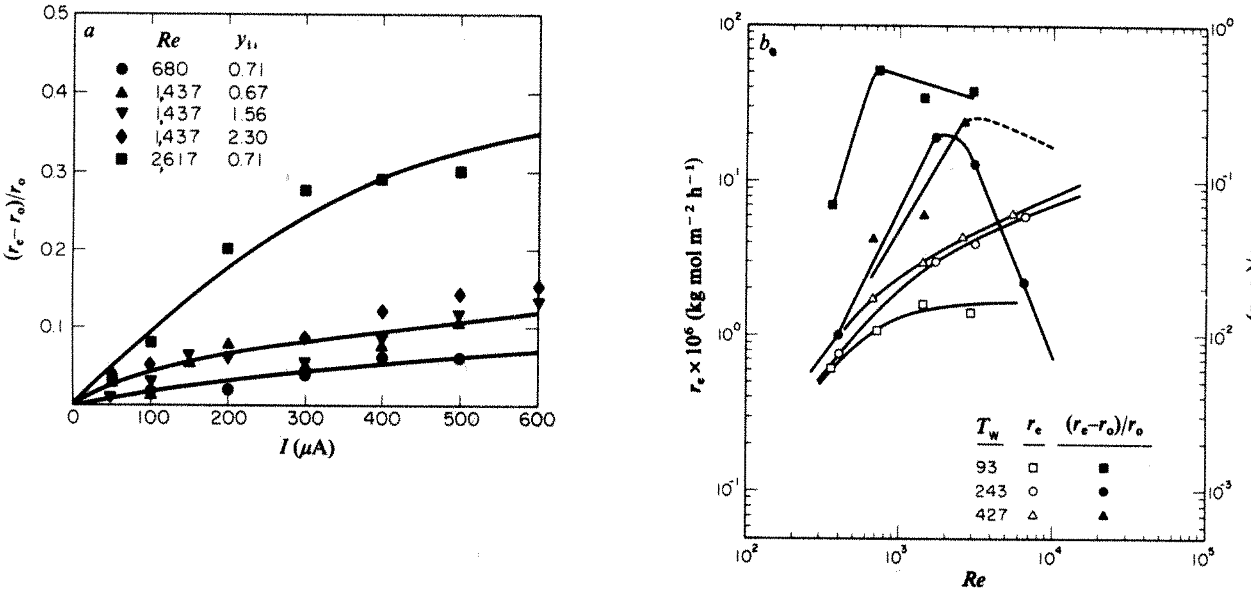

Fig. 2 *a*, Fractional increase in reaction rate as a function of corona discharge current: $T_w = 427$ °C; $T_B = 263$ °C. *b*, Reaction rate and augmented reaction rate as functions of Reynolds number at constant corona current ($I = 300$ μA).

particularly evident at $T_w = 243$ °C, where the augmented reaction rate rapidly approaches the value for no corona current as the Reynolds number attain values normally associated with turbulent tube flows. Note also that fractional augmentation of reaction rate at a given Reynolds number exhibits a complex dependence on wall temperature. This is shown in Fig. 2*b* where $(r_e - r_0)/r_0$ for $T_w = 93$ °C is much larger than at $T_w = 243$ °C and 427 °C even though the overall reaction rate is lower. Additional experiments are needed, however, to determine completely the dependence of augmented reaction rates on *I*, *Re* and T_w.

The degree to which molecular diffusion limits the overall reaction rate is expressed in terms of the effective first-order rate constant derived from a one-dimensional analysis of the overall reaction rate data[9,10]. Rate constants without corona discharge present exhibit a transition from mixed kinetics at $T_w = 93$ °C to nearly diffusion controlled at $T_w = 427$ °C (Fig. 3*a*). The presence of corona discharge produces the increase in rate constants as shown in Fig. 3*b* for a narrow range of Reynolds numbers. Here it is seen that, at $T_w = 427$ °C where rate constants can be interpreted as mass transfer coefficients[9,10,12], the corona discharge can produce up to a 50% augmentation in the rate constant. The error bars on each of the data points in Fig. 3*b* represent the maximum variation in the measured values.

Analysis of these experimental data has revealed no general mass transfer correlation for the data at $T_w = 243$ °C and 427 °C. Tentatively, we conclude that the augmentation of the reaction rate, or the rate constant, at these temperatures varies as $I^{1/2}$ at a constant Reynolds number. This result is reassuring in view of the work of Velkoff and Godfrey[13] and Mizushina *et al.*[14]. However, additional experiments and analysis are needed to permit a mass transfer correlation of general engineering utility. For the data in the regime of mixed kinetics, that is, $T_w = 93$ °C, it is doubtful that diffusional and kinetic effects can be separated for a wide range of corona current.

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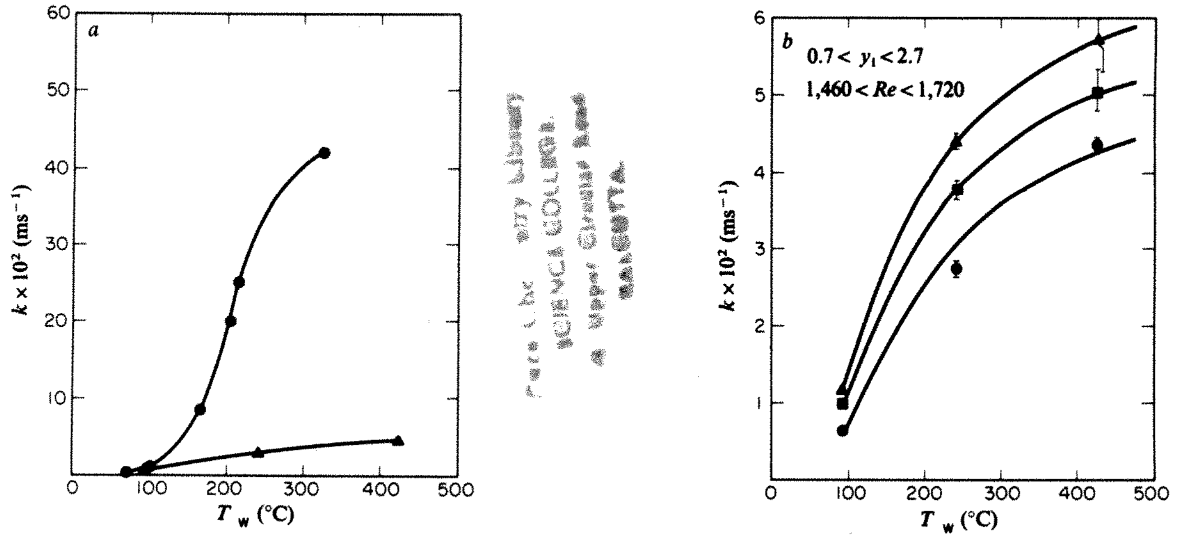

Fig. 3 *a*, Comparison of overall first-order rate constants of the present study (▲) with true rate constants (●) measured by Kulacki and Gidaspow[9]. *b*, Overall first-order rate constants determined from a one-dimensional analysis of the reaction rate data. Curves are matched by eye. ▲, $I = 500$ μA; ■, $I = 300$ μA; ●, $I = 0$.

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Seasonal change in the flux of organic carbon to the deep Sargasso Sea

W. G. Deuser & E. H. Ross

Woods Hole Oceanographic Institution, Woods Hole,
Massachusetts 02543

Recent evidence[1–5] has suggested that diurnal and annual periodicities affect the deep sea which traditionally had been thought to be the least variable environment at the surface of the Earth[6,7]. We report here what we believe to be the first direct measurements of seasonal change in the total flux of particles <13 cm and in the flux of organic carbon associated with those particles. The change seems to be related to seasonal change in the primary productivity of the surface water.

Between April 1978 and July 1979 a series of samples of particulate matter were collected in a sediment trap similar to the type described by Honjo[8]. The trap has a collecting cross-section of 1.54 m^2 and could accept particles <13 cm. The largest particles collected, in fact, were about 10 mm in length. Collection periods ranged from 60 to 68 days. The trap was moored 1,000 m above the sea floor and intercepted the particle flux at a depth of 3,200 ± 100 m. It did not collect during its descent or its ascent. The collection site was 25 nautical miles south-east of Bermuda. The experiment was not designed primarily to measure the organic carbon flux. No preservative or poison was contained in the collection cup of the sediment trap, but their absence is not expected to cause significant loss of organic carbon at that depth within 2-month collection periods[8].

Data on the total particle catch, on the mass distribution of particles smaller and greater than 125 μm, and on organic carbon content are shown in Fig. 1. The data are averaged over the collection periods. The last two sampling periods cover approximately the same time of year as the first two. For comparison, Fig. 1 also shows the monthly averages of the daily net primary productivity measured by Menzel and Ryther[9] between December 1957 and April 1960. Those measurements were made at a location 10 miles from the mooring site of our sediment trap.

Figure 1 shows: (1) fluctuation of a factor 2.75 in the amount of particulate matter collected per unit time; (2) relatively smooth variations of the amounts through the 16 months, with highs in April/May and lows in the summer months; (3) similar variations in the size fractions and the organic carbon as in the total amounts; (4) an indication of some year-to-year variation, with the 1979 high about 1.7 times as high as the 1978 one; and (5) similar locations of the highs and lows in the particulate flux and in the primary productivity.

Most of the material collected in the trap was of biogenic origin, with clay and other non-biogenic mineral particles representing only a minor part of the <37-μm size fraction. Most of the material consisted of whole individuals or fragments of Pteropoda, Foraminifera, Radiolaria, Gastropoda, diatoms and coccoliths, with occasional otoliths, squid beaks, fragments of bryozoan colonies and plant debris. Faecal pellets in various states of preservation were common and may have been more so in the undisturbed suspension in the collection cup of the trap. The organic carbon content was determined only on subsamples of the <125-μm fraction because of the greater heterogeneity of the coarser material and the destructive nature of the measurement. However, the <125-μm fraction comprised 82 ± 4% of the total samples and we do not believe that extrapolation of the organic carbon content as measured on that fraction over the entire samples introduces a significant error. The organic carbon in all samples had a rather uniform δ^{13}C of -21.4 ± 0.3‰ relative to the PDB standard, a typical value of open-ocean particulate organic carbon[10].

The data suggest that the flux to the deep sea of particles and of organic carbon in all but very large particles not captured by the trap is not uniform with time. There is indication of a seasonal cycle in that supply which, in the positions of its maximum and minimum, is similar to the seasonal cycle in primary production measured by Menzel and Ryther[9]. Unless the food supply to the deep-sea benthos consists predominantly of the very large particles not collected by a sediment trap of this type, these data represent the first indication of fluctuations in the supply of organic matter to the deep sea remote from major land areas. Seasonal variations in the standing crop of organic aggregates had been noted earlier by Riley *et al.*[11]. The highs and lows in the supply are apparently linked to the highs and lows of primary production in the surface waters and thereby point towards a possible link between seasonality of surface productivity and seasonal breeding and growth cycles in the deep sea[2–5]. Because productivity was not measured simultaneously with our trap collections, and our collections extended over two-month periods, we cannot see what delay there is between the peak in surface productivity and the peak in arrival of particulate matter in deep water. The biweekly measurements of Menzel and Ryther showed peaks in primary production from February to April; our measurements of the particulate flux peak in April/May but the peaks in the deep water may also extend back into March which was not sampled in either year. The data suggest that seasonality in the organic carbon flux is not wiped out at a depth of 3,200 m, indicating rapid settling of the greater part of the organic matter, much of it probably in the form of faecal pellets[8,12,13].

The year-to-year variation is of the same order as that observed by Menzel and Ryther[9], but we may have missed the 1978 peak just before our sampling programme began.

It is possible that the observed fluctuations are due to the trap collecting particles carried in by currents from different directions at different times of the year. If this mechanism operates it does not seem to do so on a very large geographical scale. The material must have come from an area with very similar seasonal pulsation as is shown by the correspondence with the primary production cycle. We have also observed a similar correspondence in the succession of population peaks of planktonic foraminiferal species in the surface water[14] and the arrival of those peaks in the sediment trap. Any lateral influx of particles into the water surrounding the sediment trap does not apparently eliminate the seasonal signal.

The variations in the particle flux shown in Fig. 1 might also have been caused by varying particle trapping efficiency of a uniform flux if there were a seasonally changing current regime at the depth of the trap. Gardner[15] has shown that funnel-shaped traps with baffles at the top, similar to the design used in this study, have an overall trapping efficiency of 70–90% for

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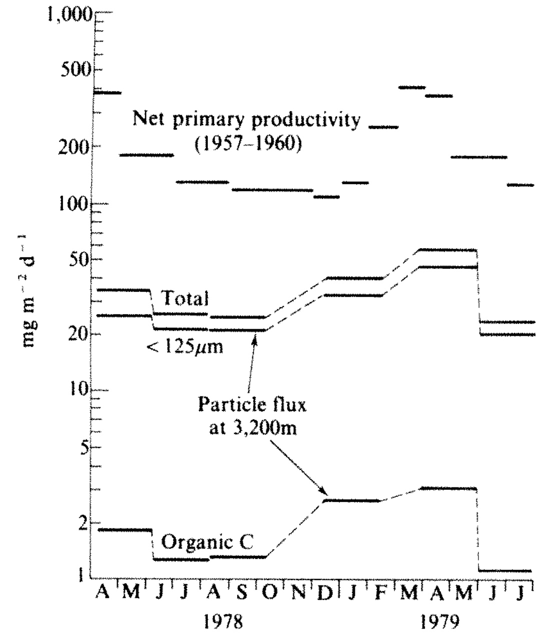

Fig. 1 Comparison of monthly averages of daily net primary productivity (in terms of carbon) for the years 1957–60 (ref. 9) with the average daily yields of total sediment (dry weight) and particulate organic carbon during six recent 2-month sampling periods by a sediment trap. The measurements refer to locations 15 and 25 miles, respectively, south-east of Bermuda. The trap was placed 3,200 m below the surface and 1,000 m above the sea floor.

particles <25 μm in horizontal flows up to 9 cm s^{-1}. For larger particles the efficiency should be greater. Gardner also showed that the efficiency of particle retention in the trap varies with flow speed and angle of attack of the flow. While we have no current data from the mooring site and depth, continuous measurements at a depth of 4,018 m, 27 miles north-northwest of Bermuda (N. Hogg, personal communication) show no indication of a seasonal signal in either speed or direction. The period covered by that measurement series was April 1975 to January 1976. Current speeds were generally <15 cm s^{-1} and 70% of the time <7.5 cm s^{-1}. There is no evidence that the current regime at the trap site should be significantly different. Neither is there any theoretical reason nor experimental evidence for seasonal current changes at that depth anywhere in the North Atlantic. Therefore, we consider it unlikely that the flux variations we have observed are a sampling artefact. We cannot, however, completely dismiss the possibility that the observed fluctuations are random rather than seasonal. Observations over a much longer time span are needed for a proper assessment of that possibility.

It is interesting to compare the range and means of the net primary production[9] and of the arrival of organic carbon at a depth of 3,200 m at very nearly the same location. Menzel and Ryther measured net primary production in the range 100–800 mg C m^{-2} d^{-1} with a mean around 200 mg. Our measurements at 3,200 m show fluxes ranging from 1 to 3 mg with a mean close to 2 mg. It thus seems that 1% of the net production at the surface penetrates beyond 3,000 m with the small-particle flux, a figure which agrees with many earlier deductions[16] as well as with *in situ* respiration measurements of benthic communities at comparable depths[17].

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Direct measurement of the attenuation of ocean waves by pack ice

Vernon A. Squire & Stuart C. Moore

Scott Polar Research Institute, University of Cambridge, UK

Early experimental work in the Antarctic[1] using a ship-borne wave recorder has shown that pack ice can significantly attenuate incoming ocean waves, particularly those of shorter period. Wadhams[2,3] has subsequently reported similar wave decay through Arctic ice in data obtained remotely by means of airborne laser profiling and inverted echo sounding from a submarine. The two predominant mechanisms by which the decay can occur—scattering by individual ice floes and energy loss by creep within each flexing floe—have been studied theoretically by Wadhams[4,5], Squire[6,7], and Squire and Allan[8]. We present here the results of further experiments to measure wave decay which took place in the Bering Sea during spring 1979 from the research ship Surveyor of the National Oceanic and Atmospheric Administration[9]. During the cruise, oceanographic, meteorological and remote sensing data were also collected[10,11]. The results show the energy decay of waves in pack ice to be exponential with an attenuation coefficient which increases with decreasing wave period.

The attenuation experiment comprised eight floe stations of similar size and thickness, which lay along a line parallel to the direction of the observed principal swell (the attenuation line). They were chosen so that their separation increased away from the ice edge (Fig. 1). The seven floes within the ice pack were visited using the ship's Bell 206 helicopter equipped with a GNS-500A global navigation system which had been preprogrammed from Loran-C on Surveyor. Distances calculated from position fixes should therefore be good to within 0.2 km (Table 1). The first floe, situated on the ice edge itself ~1 km from Surveyor, was reached using the ship's whaleboat. The heaving motion of each floe was measured using a Schaevitz-EM vertical accelerometer. For logistic reasons we were not able to instrument floes simultaneously so that the complete experiment lasted about eight hours. Ideally such an experiment should take place over a very short time so that waves incident on the ice cover may be assumed to be statistically stationary. To

Table 1 Position of ice floes along attenuation line

Floe station	1	2	3	4	5	6	7	8
Distance from ice edge (km)	0	0.7	2.2	5.3	10.3	18.9	38.6	65.1

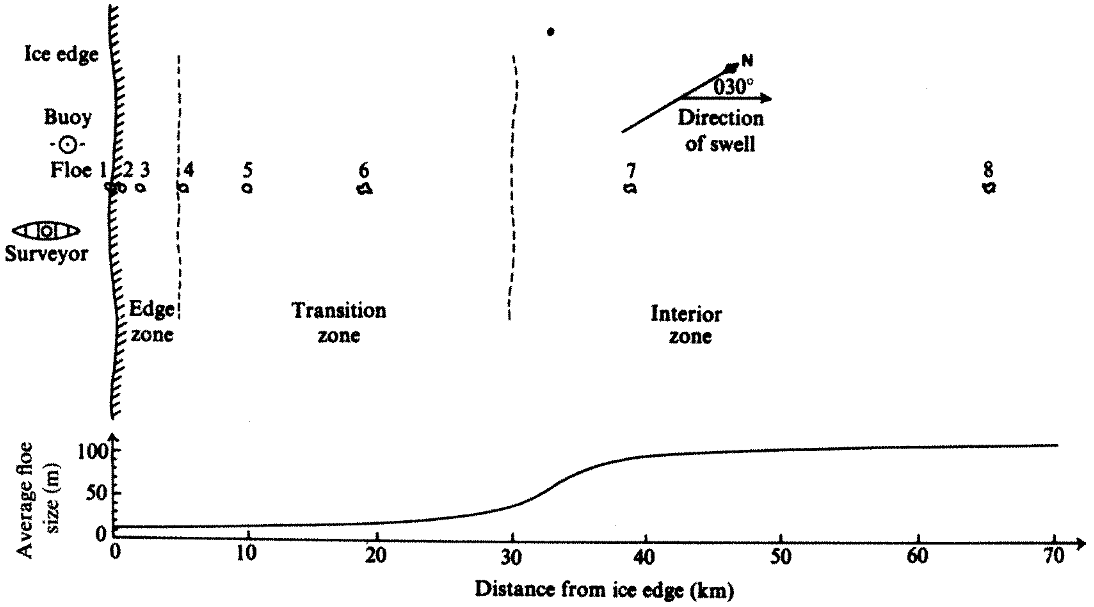

Fig. 1 Schematic diagram of attenuation experiment.

overcome this problem a Waverider buoy was deployed from Surveyor to monitor the open-water wave spectrum.

Routine ice observations were carried out during each helicopter flight. The ice margin was diffuse with an average concentration of four Oktas for tens of kilometres into the ice cover with occasional isolated bands of higher concentration. Floe diameter increased gradually from 10 m at the ice edge to 40 m some 30 km in. At 30 km, the floe size increased abruptly to more than 100 m in diameter, the typical size thereafter. From the air the ice cover could be divided into three distinct zones made up of floes of quite different character: the edge zone, which stretched from the ice edge some 5 km into the pack; the transition zone, which stretched a further 25 km; and the interior zone. This zonal structure had been observed earlier in the field trip and was the reason behind our choice of floe separation. The edge zone was made up of floes which had been buffeted together in waves of sufficient intensity to ridge, raft and fracture the ice. Floes were typically 10 m across, of uneven thickness, with heavily rafted perimeters. Floes within the transition zone were much smoother since the incoming waves had lost much of their energy in the extremely active edge zone. The penetrating waves were still able to fracture the ice, however, and the average floe diameter remained 10 m. Further into the ice cover, the incoming swell had been attenuated to such an extent that little or no fracturing took place and floes in the interior zone were typically smooth and >100 m across.

All the floes visited were between 10 and 20 m across, less than 0.5 m thick and relatively smooth. CTD casts had shown the underlying seawater to be well-mixed and well above the freezing point, and this together with several days of warm weather, had left the ice in a mushy and decaying condition. Twenty minutes of wave data were collected on each of the floe stations and it was noted that there were at least two components of swell present. There seemed to be a noticeable attenuation between floes but at this stage we could not be sure that the change in amplitude was not due to a decline in the open-water wave forcing. The wave records obtained at each site were digitised and, together with the Waverider buoy data, were calibrated and transferred to IBM digital tape for further analysis. All data processing was carried out on the IBM 370 at Cambridge University using the FFT/graphics package developed by the Sea Ice Group at the Scott Polar Research Institute (SPRI).

Table 2 The attenuation coefficients for energy decay at various central wave periods

Central period (s)	Attenuation coefficient ($m^{-1} \times 10^{-4}$)
12.2	0.272±0.054
9.4	0.438±0.036
7.6	0.855±0.049
6.4	1.087±0.037
5.5	1.214±0.192

The Waverider buoy data are presented in Fig. 2. Each power spectrum represents a 20-min sample of the open-sea wave conditions during the experiment. Records are between 1 and 2 h apart. Within the statistical errors generated by a frequency domain analysis[12], the smoothed power spectra show the wave conditions off the ice edge to be stable throughout the day. This being so, the wave forcing for the attenuation run may be assumed stationary and any comparison between wave data from individual ice floes is valid.

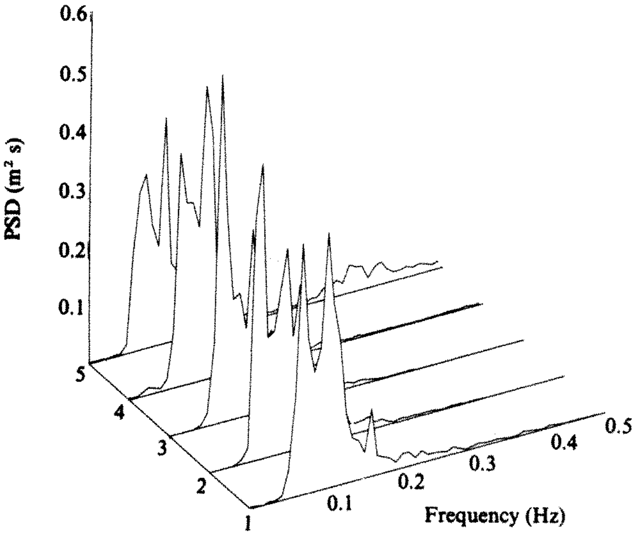

Fig. 2 Three-dimensional graph showing evolution of power spectra from Waverider buoy during the experiment.

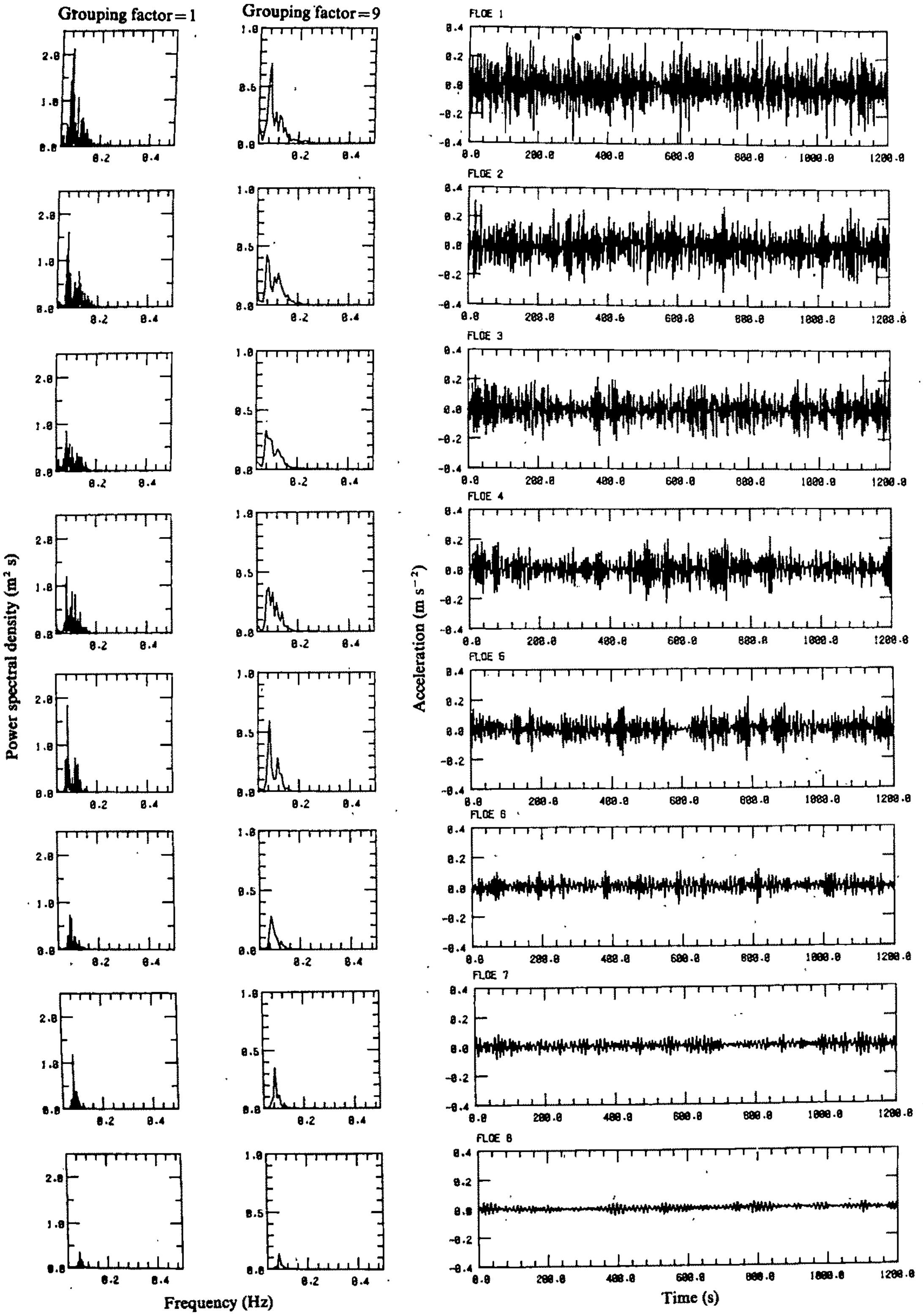

Fig. 3 The attenuation run showing time series at each floe and corresponding power spectra.

Figure 3 shows the time series and the unsmoothed and smoothed power spectra for the wave data recorded at each floe station. All records are 20 min (1,200 s) long and are plotted to the same scale so that a direct comparison may be made. Each time series represents the vertical acceleration at a point along the attenuation line. There is a clear attenuation with distance from the ice edge together with a noticeable filtering of the incoming waves in favour of longer wavelengths. The power spectra confirm these observations. Each pair of spectra, derived from the corresponding time series and corrected for acceleration, shows the power spectral density (PSD) as a function of frequency. The first spectrum of the pair is unsmoothed and is presented for completeness. The second has been frequency smoothed with a grouping factor of nine. There is a striking decay in the available wave energy as the reader proceeds from floe to floe. Furthermore,

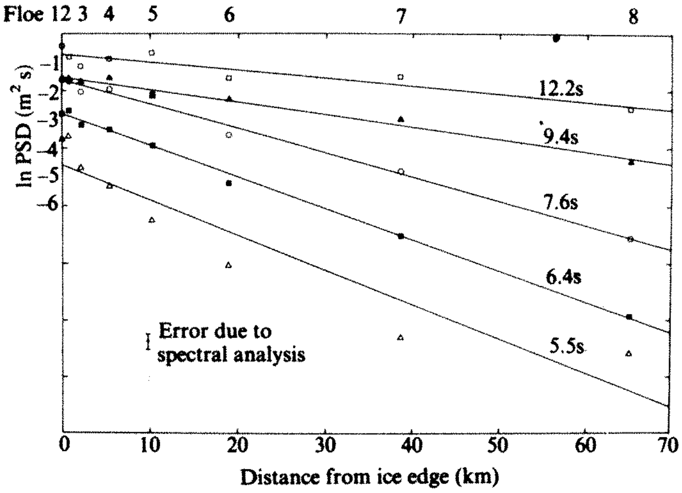

Fig. 4 Energy decay as a function of wave period.

waves of shorter period are attenuated more rapidly than long waves.

From the smoothed spectra, five central wave periods were selected at which significant wave energy was present. Figure 4 shows these wave energies plotted against distance from the ice edge. The energy decay is seen to be approximately exponential with the attenuation coefficient a function of wave period (Table 2). The line corresponding to a period of 5.5 s shows the most scatter. This is believed to be due to an increase in energy at shorter periods occurring late in the day (see Fig. 2). If the 5.5 s point for floe 8 is assumed to be an overestimate and is neglected, the best straight line between the remaining seven stations gives an attenuation coefficient of $(1.824 \pm 0.183) \times 10^{-4}\ \mathrm{m}^{-1}$. With this revised value for the energy decay at 5.5 s, the data fit the empirical inverse square relationship between attenuation coefficient and wave period found by Wadhams[2]. Similar studies carried out off the coast of East Greenland[13] by SPRI, the Cavendish Laboratory at the University of Cambridge, and the Technical University of Denmark promise attenuation runs for thicker floes and longer wave periods.

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KBS Tuff dating and geochronology of tuffaceous sediments in the Koobi Fora and Shungura Formations, East Africa

R. E. Drake*, G. H. Curtis*, T. E. Cerling†,
B. W. Cerling† & J. Hampel*

*Department of Geology and Geophysics, University of California, Berkeley, California 94720
†Department of Geology and Geophysics, University of Utah, Salt Lake City, Utah 84321

Plio–Pleistocene lacustrine and fluvial sediments of the Koobi Fora Formation along the east shore of Lake Turkana in northern Kenya (see Fig. 1) and thicker fluvial sediments of the Shungura Formation in the Lower Omo River Basin 100 km to the north have become widely recognised because of their rich hominid and artefact assemblages, together with associated vertebrate fauna[1–3]. Over the past decade, tuffaceous horizons within these two sedimentary formations have been mapped[4,5] and dated by K–Ar[6–11] and fission-track techniques[12] to provide chronologic control supporting studies of hominid and faunal evolution and archaeology. We report here new ^{40}K–^{40}Ar dates and revised values for previously published dates which give a mean age of 1.8±0.1 Myr for the KBS Tuff. This estimate suggests contemporaneity between the KBS Tuff and Tuff Units H2 and H4 in the Shungura Formation, lower Omo River Basin, Ethiopia, and with Bed I at Olduvai Gorge.

Although isotope dates for most of these tuffaceous horizons have been stratigraphically and palaeontologically consistent, some have remained uncertain. Of these, the KBS Tuff of the Koobi Fora Formation has been the most controversial, particularly because of its importance in placing an upper age limit on the important hominid skull KNM-ER-1470 with its associated vertebrate fauna, and on artefacts embedded in the tuff[13,14].

In 1975, we reported ^{40}K–^{40}Ar dates indicating the KBS Tuff and its correlative horizons contained at least three pumice populations with two different ages[9]. All dates from Area 105 (see Fig. 1) averaged 1.6 Myr, while those from Area 131 averaged 1.8 Myr. Both dates were significantly younger than the previously assigned age of 2.61 Myr based on a ^{40}Ar–^{39}Ar incremental heating date[6]. Even though the 2.61 Myr date was subsequently revised to 2.42 Myr (ref. 10), in apparent agreement with newly obtained fission-track dates on zircons separated from KBS pumices[12], a substantial age discrepancy still remained between our conventional ^{40}K–^{40}Ar results and those obtained by ^{40}Ar–^{39}Ar and fission-track dating techniques.

To resolve this problem, we dated a new group of KBS pumice samples that were collected in 1977. All of these new dates fell in the 1.8 Myr range, and none reproduced our previous 1.6 Myr dates. This result led to the discovery of a systematic error in potassium analyses for all KBS samples which had a given 1.6 Myr date. This error was traced to a defective balance. Recalculation using revised potassium contents obtained by re-analysis of the originally dated material increased all of the 1.6 Myr dates to 1.8 Myr, bringing them into agreement with the new dates, and completely eliminating the evidence of any KBS pumice 1.6 Myr old (see Fig. 2.)

With this revision, we now have 22 KBS dates; 16 on feldspars and 6 on glasses that were separated from a total of 13 different pumice samples collected within the KBS Tuff in Areas 131, 105 and 112. Fig. 2 shows that statistically, these dates give a mean of 1.8±0.1 Myr at the 95% confidence level. For correlation and interpretation, the dates of all of the pumices so far sampled must be treated as being indistinguishable in age, even though several geochemically distinct pumice types representing separate eruptive events occur together in the KBS Tuff horizon. As we can distinguish dates that are >100,000 yr apart,

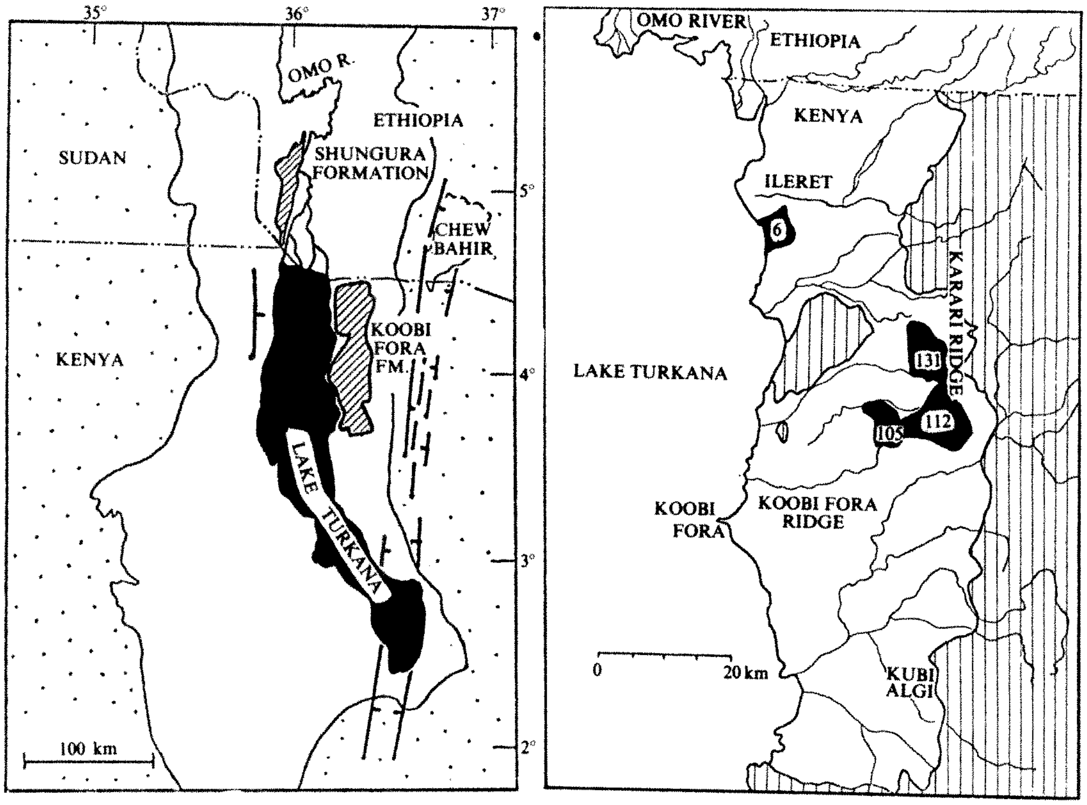

Fig. 1 Maps showing sample localities in the Koobi Fora Formation east of Lake Turkana and their geographic relation to the Shungura Formation in the lower Omo River Basin.

it seems reasonable to assume that several eruptive events which led to the accumulation of the various pumice fragments in the KBS Tuff took place within this time interval, and may contribute to the scatter of dates about the mean.

In 1970, the first K-Ar dates from the KBS Tuff were obtained on feldspars by Fitch and Miller using ^{40}Ar–^{39}Ar incremental heating and total degassing procedures[6]. Further work produced dates ranging from 0.52 to >200 Myr (ref. 8). As Fitch and Miller recognised, the extremely old dates resulted from detrital contamination with ancient crystalline basement rocks. Disregarding those old dates, one is still faced with a broad range of possible dates from which 2.61 ± 0.26 Myr was originally selected as the 'best apparent age' of the KBS Tuff. The subsequent revision to 2.42 ± 0.01 Myr using a different J value[10] did not reduce this scatter. We feel that the apparent discrepancy between our ^{40}K–^{40}Ar results and the date favoured

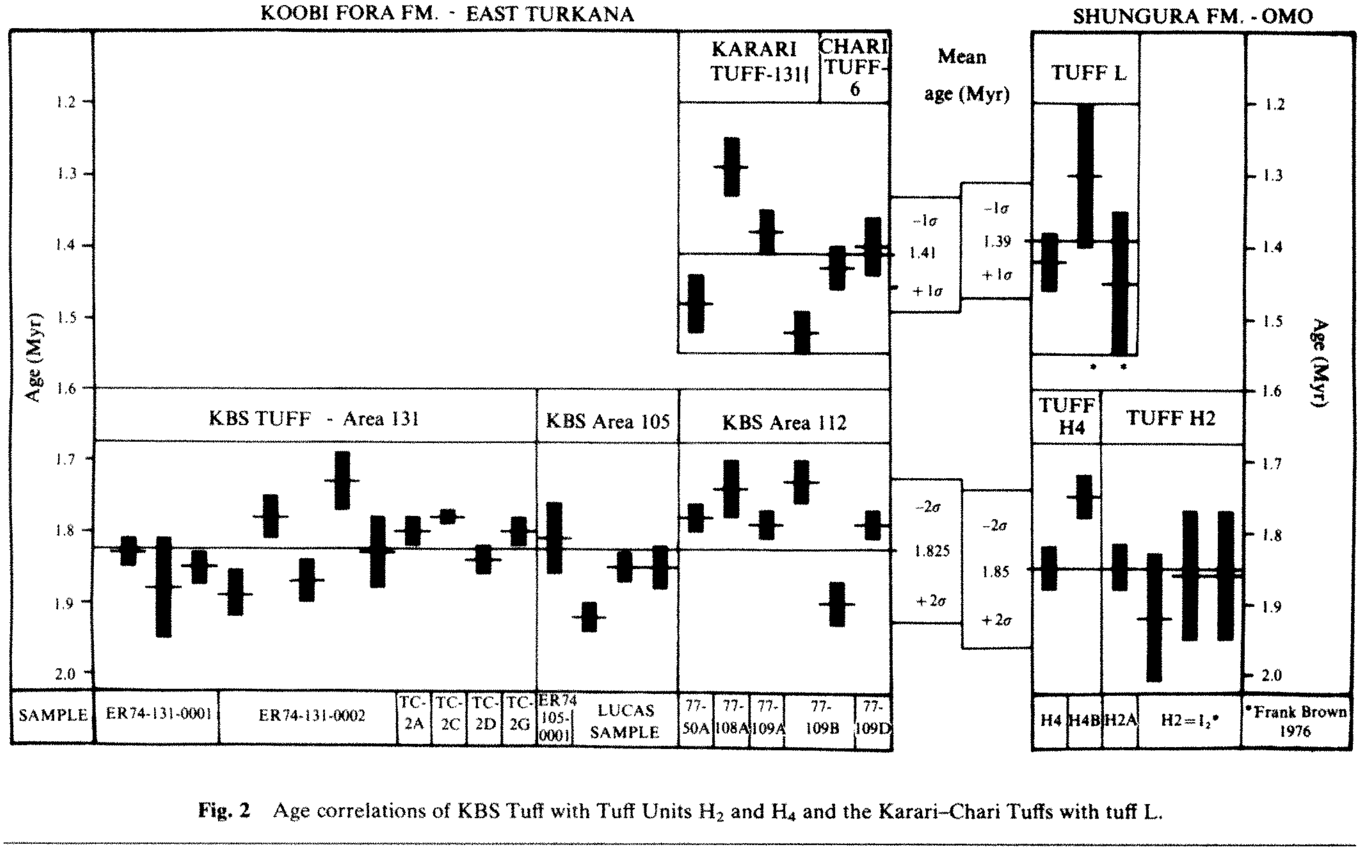

Fig. 2 Age correlations of KBS Tuff with Tuff Units H_2 and H_4 and the Karari–Chari Tuffs with tuff L.

Table 1 K–Ar analytical data

KA No.	Material	Sample wt (g)	% K	^{40}Ar radiogenic ($\times 10^{-11}$ mol g^{-1})	% ^{40}Ar atmospheric	Age (Myr)	Sample	Location
2836	sanidine	4.877	5.10*	1.62	36	1.83±0.02	KBS ER74-131-0001	Area 131
2837	glass	3.477	3.92	1.28	93	1.88±0.07		
2869	sanidine	2.156	4.60	1.47	53	1.85±0.02		
2841	sanidine	1.945	4.90*	1.61	52	1.89±0.03	KBS ER74-131-0002	
2842	sanidine	3.002	5.11	1.58	41	1.78±0.03		
2843	glass	3.514	3.48	1.13	83	1.87±0.03		
2858	glass	2.870	3.69*	1.11	81	1.73±0.04		
2862	glass	2.778	3.73*	1.18	83	1.83±0.02		
3160	sanidine	6.266	5.09	1.59	30	1.80±0.02	KBS TC-2A	
3166	sanidine	3.321	5.04	1.56	26	1.78±0.01	KBS TC-2C	
3161	sanidine	3.493	5.12	1.64	37	1.84±0.02	KBS TC-2D	
3167	sanidine	3.019	4.87	1.61	36	1.90±0.02	KBS TC-2G	
2851R	sanidine	1.940	5.55*	1.74	39	1.81±0.03	KBS ER74-105-0001	Area 105
2856	sanidine	1.736	4.70*	1.57	37	1.92±0.02	KBS Fred Lucas sample	Area 105E
2854	glass	3.558	3.89*	1.25	53	1.85±0.02		
3496	sanidine	2.253	5.11	1.64	73	1.90±0.03		
3182	sanidine	2.422	5.19	1.61	42	1.78±0.02	KBS 77-50A	Area 112
3189	sanidine	3.504	5.08	1.54	74	1.74±0.04	KBS 77-108A	'Hippo site'
3188	sanidine	2.699	4.96	1.54	33	1.79±0.02	KBS 77-109A	Area 112
3186	sanidine	4.438	4.87	1.46	59	1.73±0.03	KBS 77-109B	
3380	glass	3.010	4.04	1.33	51	1.90±0.03	KBS 77-109B	
3187	sanidine	6.142	5.12	1.59	30	1.79±0.02	KBS 77-109D	
2970	sanidine	2.339	5.06	1.62	45	1.85±0.03	H_4	Omo
3183	sanidine	1.143	5.09	1.54	60	1.75±0.03	H_{4B}	
3256	sanidine	4.291	4.93	1.59	48	1.85±0.03	H_{2A}	
3254	sanidine	2.322	3.94	0.96	71	1.42±0.04	L	
2815	sanidine	4.264	4.05	1.04	77	1.48±0.04	Karari 108-000	Area 131
2863	sanidine	1.676	4.15	0.93	82	1.29±0.04	Karari 108-0003	
2882	sanidine	2.748	4.24	1.12	36	1.52±0.03	Karari 108-0002	
2880	sanidine	3.161	4.08	0.97	41	1.37±0.03	Karari 108-0004	
2865	sanidine	1.718	3.92	0.94	59	1.38±0.03	Chari 006-0001	Area 6
2883	sanidine	1.010	3.89	0.94	75	1.40±0.04	Chari 006-0120A	

Decay constants: $\lambda_{40K\beta} = 4.962 \times 10^{-10}$ yr; $\lambda_{40Ke} + \lambda'_{40Ke} = 0.581 \times 10^{-10}$ yr

Isotopic abundance: $^{40}K = 0.01167\%\ K_{total}$

* K content reanalysed and date recomputed from 1975 published results. All 1975 dates have been recalculated using the above constants.

by Fitch and Miller arises through their selection of a particular value from a scatter. Their criteria used for selecting this date are debatable. Based on their experience of interpreting ^{40}Ar–^{39}Ar release patterns of plutonic minerals known to have been thermally overprinted, they believed they could not only see effects of similar overprinting of feldspar from KBS pumices, but could also infer a geological age for these events. Such an interpretation for KBS pumice dates is not supported, however, either by our data or by theirs. Furthermore, many of those with ^{40}Ar–^{39}Ar dating experience support the conclusions of Lanphere and Dalrymple[15] that "it may be difficult or impossible to extract geologically meaningful information from the age spectrum of a disturbed sample."

We have demonstrated the repeated concordancy of dates on glass and feldspar taken from the same pumice clasts (Table 1). As glass devitrifies and loses argon much more readily than sanidine or anorthoclase feldspar, the concordancy of dates argues strongly against thermal overprinting. Such overprinting would manifest in significantly younger dates for glass with respect to coexisting feldspar.

To interpret Fitch and Miller's ^{40}Ar–^{39}Ar incremental heating data following a thermal overprinting model requires a different history of thermal events for almost every sample dated. Only in this way can many otherwise reasonable KBS dates be disregarded. (In fact, several of Fitch and Miller's better total degassing dates and at least one ^{40}Ar–^{39}Ar age spectrum support a 1.8 Myr age.) The inability to obtain reproducible results apparently led to special interpretations which are not satisfactorily supported by the data presented. We feel, therefore, that the 1.8 Myr mean of our tightly clustered ^{40}Ar–^{40}K dates provides a better estimate of the age of the KBS Tuff than the ^{40}Ar–^{39}Ar dates selected by Fitch and Miller.

Another aspect of the dating problem was brought to light by fission-track dates on zircons[12] from two KBS pumices which gave a mean of 2.44±0.08 Myr, apparently supporting the ^{40}Ar–^{39}Ar age advocated by Fitch and Miller[10], but differing from our 1.8 Myr ^{40}K–^{40}Ar mean of 1.8 Myr.

Although the mean of five dates yielded this statistically precise value, the individual dates have much larger error estimates because fission-track counting was done only on a few zircons which have very low track densities. Hence, any minor systematic error in technique could greatly bias the age, and a statistically derived estimate of precision may not be relevant to the accuracy of the mean of these dates. If this is so, the apparent discrepancy between our ^{40}K–^{40}Ar mean date of 1.8 Myr and the fission-track mean may be resolved by increasing the fission-track confidence interval to express the possibility that systematic errors may have yielded an excessive age. This apparent discrepancy between the fission-track age estimates of Hurford, Gleadow and Naeser[12] and the mean of our 22 tightly clustered ^{40}K–^{40}Ar dates deserves investigation, but for the present we contend that our mean date is an age estimate with much more clearly defined confidence limits than the fission-track age.

The mean value of 1.8±0.1 Myr for the KBS Tuff and a mean value of 1.4±0.1 Myr for the Chari–Karari Tuffs of the Koobi

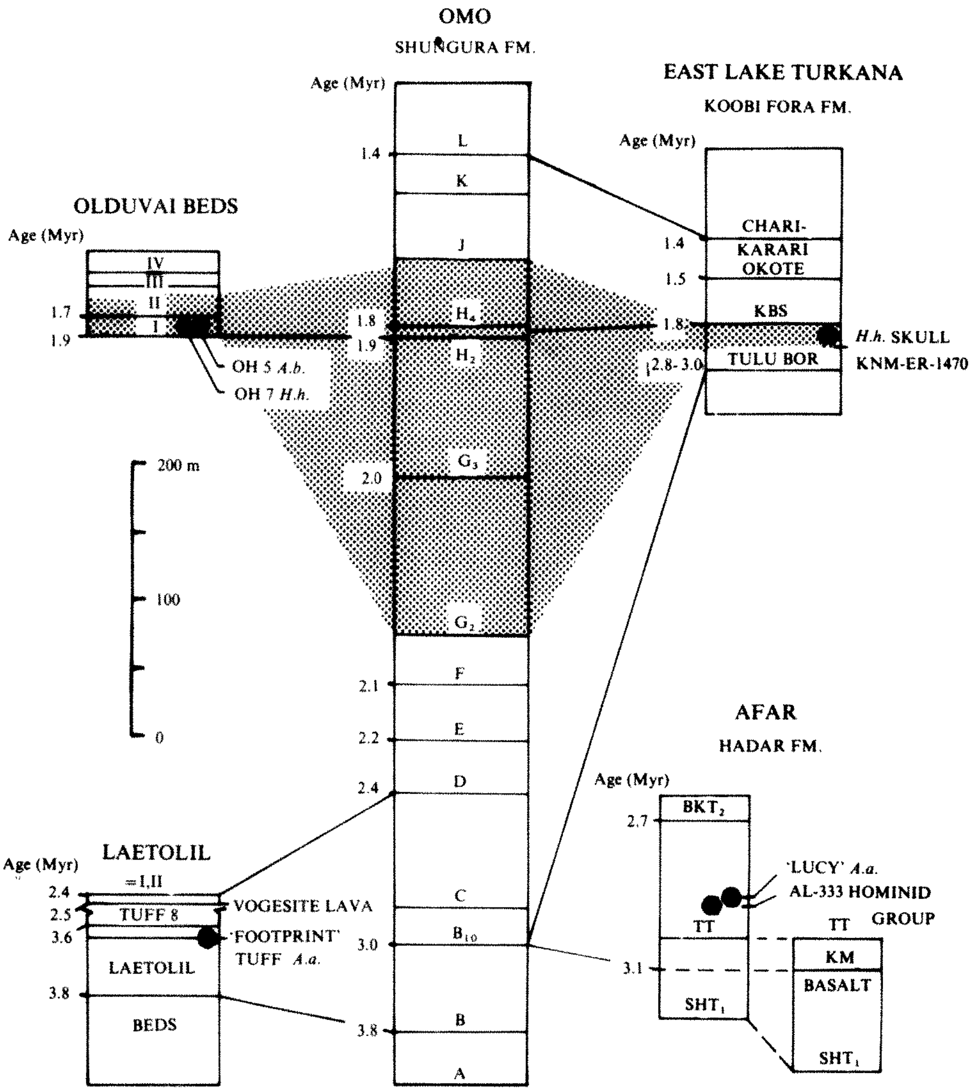

Fig. 3 Comparison of dated stratigraphic sections of the Koobi Fora Formation, Shungura Formation, Laetolil Beds, and the Hadar Formation in East Africa. The stippled area represents biostratigraphically contemporaneous units.

Fora Formation are indistinguishable from K–Ar dates on Tuff Units H_2 and H_4 and Tuff Unit L[11] respectively in the Shungura Formation (Fig. 2).

The results of extensive geochemical and geophysical analyses of pumices and tuffaceous matrix from these tuffs[19] have enabled the identification of unique pumice types in the KBS-H_2, H_4 horizons that are probably derived from the same eruptive event though they occur in sedimentary sections 100 km apart. As the Shungura Formation has yielded a long and consistent sequence of ^{40}K–^{40}Ar dates, this independent geochemical correlation of the KBS Tuff and Tuff Units H_2 and H_4 strengthens the case for a 1.8 Myr age for the KBS Tuff.

Figure 3 compares the generalised stratigraphic sections and dated horizons within the Olduvai Beds, the Shungura Formation, the Koobi Fora Formation, the Laetolil Beds, and the Hadar Formation to show the relationship of the ^{40}K–^{40}Ar time scales that have been established for each stratigraphic section[11,16–18].

Cooperative studies of the taxonomic composition of fossil vertebrate assemblages in the beds immediately below the KBS Tuff, between Tuffs Gi and J in the Shungura Formation and in Bed I at Olduvai Gorge suggest that all three are biostratigraphically indistinguishable units[1,2] as shown by the stippled area of Fig. 3. Further detailed studies of the stage of evolution of certain rapidly changing lineages, especially among the Suidae also suggest contemporaneity (see refs 20, 21).

Bed I at Olduvai Gorge has been dated by numerous ^{40}K–^{40}Ar dates as between 1.7 and 1.9 Myr (ref. 17). Member G between Tuff Units G and H in the Shungura Formation is dated by ^{40}K–^{40}Ar as between 1.8 and 2.0 Myr (ref. 11). These beds and those below the KBS Tuff exhibit a palaeomagnetic stratigraphy consistent with an Olduvai event age between 1.76 and 1.91 Myr (refs 22, 23). We consider it unlikely, therefore, that the KBS samples which also yield the same ^{40}K–^{40}Ar dates as the Olduvai and Omo localities could have been subjected to overprinting or other disturbances as are postulated by Fitch and Miller.

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Wind velocities determined from the surface textures of sand grains

David Krinsley & William Wellendorf

Department of Geology, Arizona State University, Tempe, Arizona 85281

Krinsley and Smalley[1] suggested that 'upturned plates', a surface texture that is represented on quartz sand surfaces by a series of thin parallel plates, either continuous or discontinuous parallel to $r(10\bar{1}1)$ and $z(01\bar{1}1)$, cause the frosting observed on desert sand grains. As these plates depend on the extent of physical abrasion[2], the spacing between individual plates might be related to the velocity of the wind which last transported the grains. The technique described here attempts to relate experimental and modern aeolian sand grain surface textures to wind velocity via scanning electron microscopy (SEM)[3].

To test this hypothesis, we examined crushed Brazilian quartz abraded in a paddle wheel device at four effective wind velocities: 8, 24, 32 and 40 m s^{-1} (ref. 4). Two modern Saharan desert sands where the average wind velocity was known from meterological data, and a Permian eolian sand were also studied. The paddle wheel apparatus was driven by a variable speed motor that moves particles at a given rate and causes particle self abrasion; the apparatus was constructed to simulate aeolian action on Mars, as previous devices could not operate at the low pressures and high velocities presumably present on Mars[4]. At normal pressures and velocities, surface textures on experimental sand grains were identical to those produced by other devices used to simulate natural aeolian processes[5]. Upturned plates on grains were photographed using SEM; stereo photogrammetry was then used to measure separation between the plates.

Plate spacing measurements on the experimental samples (175–355 μm diameter) exhibit three significant characteristics: (1) spacing is constant across individual grain surfaces; (2) spacing on grains abraded at similar velocities is reasonably constant; and (3) grain size has little effect on spacing (two grain sizes averaging 200 and 300 μm diameter were used).

Plate spacing on the 20 experimentally produced aeolian grains was measured using stereo microscopy (Table 1). The results of a regression analysis of the plate spacing (y) plotted against the wind velocity (x) for this experimental material (Fig. 1) show that the best fit ($r = 0.94$) line for these data is described by the polynomial expression:

$$y = 0.000032x^3 - 0.0017x^2 + 0.0364x + 0.0016 \qquad (1)$$

The curve representing this equation is characterised by three zones (delineated by two slope changes) which were observed in the Sahara desert[6–8].

The first zone (from 0 to 7 m s^{-1}) is the velocity range in which grains have lost all of their sharp edges and have begun to round. The presence of frosting on edges indicates that these areas were the most susceptible to abrasion. Sand grains at this range of wind velocities have been observed to be mobile for only short distances in deserts.

The second zone extends from 7 to 27 m s^{-1}; the flattening of the curve here suggests that physical changes due to abrasion are linearly related to wind velocity. The calculated average daily wind velocity is from 5 to 8 m s^{-1} in the Sahara[6] and it has been observed that sand clouds form and sand actively migrates across the desert at velocities of 10–13 m s^{-1}; sandstorms begin to form at velocities from 13.7 to 15.7 m s^{-1} (refs 7, 8). Note that the maximum continuous wind velocities observed in the Sahara are represented in this zone.

The third zone (no measurements have been continuously recorded above 27 m s^{-1} shown on the curve ranges from 27 to 40 m s^{-1}. Here the slope increases very rapidly, suggesting rapid physical changes on sand grain surfaces; these have been observed with SEM on individual sand grains abraded at the two highest velocities (32 and 40 m s^{-1}). In this range, grain size seems to decrease rapidly; the grains become more spherical, and plastic deformation and/or partial melting may occur on surfaces[4].

Micron-sized quartz particles, after abrasion at 27–40 m s^{-1}, are structurally deformed and SEM observations indicate that rounding and smoothing occur on quartz sand grains at these velocities[4]. Calculations also show that the collision between two quartz grains at 15 m s^{-1} would raise the temperature of a microscopic feature (~5 μm diameter) on their surfaces to above the melting temperature of quartz[4].

Extrapolation of the curve past 40 m^{-1} suggests that it becomes asymptotic at some higher velocity. This indicates that there may be a limit or terminal velocity where either grains achieve total sphericity or break down into silt and clay-size fragments. The large population of fine silt-sized debris in the 40 m s^{-1} sample suggests that this limit probably results in grain destruction ('kamikaze grains' as in ref. 9), and not perfect sphericity. Note that when the fine silt particles from the 40 m^{-1} sample are studied at high magnification, some of them exhibit a finely abraded and rounded surface while other surfaces display conchoidal fracture. Thus, these particles may represent portions of rounded and frosted grains which subequently have been destroyed during the experiment.

Photomicrographs of 10 modern Saharan dune sand grains were studied (Table 2) first to determine if plate spacing could be measured on naturally occurring sand grains, and second to obtain a reasonable approximation of the maximum wind velocity which last moved them.

The Libyan grains fall into the lowest velocity zone; they were collected from an inactive interdune area. Solution and/or precipitation and small bits of debris sticking to grain surfaces suggest that the grains were not being actively abraded at the time of collection. The wind conditions of this sub-environment (5–8 m s^{-1}, (ref. 8)) are comparable to those calculated directly from aeolian plate spacings (6.33 ± 0.04 m s^{-1}, Table 2).

The Algerian sand was collected from an active dune. Here, solution and/or precipitation features can be recognised only in depressions on the grains with clean surfaces indicating that they have undergone recent active abrasion. The calculated wind velocity for this sample (13.53 ± 0.05 m s^{-1}) lies well within the values observed by Bagnold for actively saltating grains (10–15.7 m s^{-1}).

Plate spacing on Permian aeolian sands was then examined (Table 2). The Permian sandstone deposits of County Durham, UK have been interpreted as a hot, dry, Saharan type of aeolian deposit[10]. Minor quartz overgrowths and precipitation features are present, but do not obscure grain surfaces. Some of these features may even aid plate measurement; the aeolian plates seem to act as a growth site for secondary quartz. Measurements

Table 1 Measured average aeolian plate spacings from experimentally abraided Brazilian quartz grains

Velocity (m s^{-1})	No. of grains	No. of measurements	Grain size range (μm)	Average spacing (μm)
8	5	98	175–355	0.207 ± 0.041
24	5	78	175–355	0.338 ± 0.048
32	5	88	175–355	0.490 ± 0.073
40	5	71	175–355	0.777 ± 0.130

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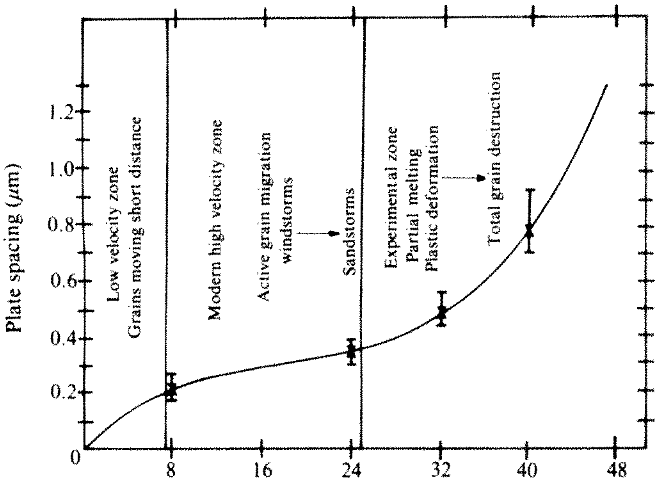

Fig. 1 Comparison between changes in slope of experimental data and wind velocity for various types of sand grain movements observed in deserts.

of plate spacing were substituted in the regression equation; the calculated wind velocity is 29 m s^{-1}, a value slightly higher than the strongest continuous wind velocities observed in the modern Sahara (22 m s^{-1} (ref. 6)). If the technique is valid, these sands could have been transported and deposited in a hot arid Saharan type desert by winds of higher velocities than those in the modern Sahara.

If the time required to remove a thickness equal to the relief of the plates is short enough, the spacing measured will represent the final abrasion episode. If the time is measured in days, weeks, or months, then the spacing must represent some average value related to the velocity of a number of storms.

Crushed Brazilian quartz averaging ~500 μm in diameter was abraded at a velocity of 8 m s^{-1} for three hours in the paddle wheel device. The grains were then removed and studied with the scanning electron microscope; >80% of all surfaces were covered with small abrasion markings. Upturned plates constituted a large portion of the abraded surfaces. Three hours is probably the maximum abrasion time necessary to cover almost completely the surface with abrasion markings; the only remaining non-abraded areas were those that were at least 5 μm below the general surface and thus were protected from immediate abrasion. Although the original starting material was fairly large and quite angular, the experiment suggests that times of the order of minutes or hours in a sandstorm would resurface natural sand grains.

Additionally, 500 g of natural quartz sand (sieved to between 125 and 180 μm) was fed into an aeolian whirling arm device[11] and used to abrade, in an aeolian mode, six quartz flats at 20 m s^{-1}. The sand stream struck the targets perpendicular to their surfaces for 23 min with 16 g of sand actually striking each target. Note that each grain of sand struck each flat only once. Finally the targets were weighed to an accuracy of five places before and after bombardment.

The time needed to remove completely a layer of quartz the height of an average plate (0.5 μm) was calculated and was 29 min. This is probably the maximum time needed to remove a significant fraction of the old plates and produce new ones; thus in a very short time a high velocity wind could reset the spacing over a large portion of grain surfaces. The experiment, of course, was conducted with a fixed target; natural aeolian grains are round and rotate, which would tend to create an isotropically eroded surface similar to the experimental targets. The two experiments give order of magnitude results, but even if the final answer were three times as large, plate spacing on aeolian grains probably represents the final windstorm velocity to which the grains were subjected.

Table 2 Sample locations, plate spacing and calculated wind velocities for Saharan and Permian sands

Sample location	No. of grains	No. of measurements	Average spacing (μm)	Calculated wind velocities (using equation (1))
Sehba Sand Sea, Libya (Sahara desert)	5	92	0.172 ± 0.045	6.33 ± 0.04 m s^{-1}
Great Western Erg, Algeria (Sahara desert)	5	102	0.262 ± 0.039	13.53 ± 0.05 m s^{-1}
Permian sandstone County Durham, UK	5	100	0.354 ± 0.047	29.34 ± 0.04 m s^{-1}

We stress that plate spacing results from the last abrasion event that occurred on a given sand grain; a change in velocity is assumed to eliminate previous plate spacing imprints and to superimpose the most recent velocity spacing on surfaces. The ability to determine ancient wind velocities could aid further understanding of the paleogeography and paleoclimatology of the vast continental aeolian sand deposits preserved in the geologic record. This research was supported by NASA consortium agreement 2-0R035-801 and 2-0R035-901, Office of Planetary Geology. We thank Paul Spudis and Alan Peterfreund for reviewing this manuscript, also Michael Malin and Steve Williams for technical assistance.

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Why do Bedouins wear black robes in hot deserts?

Amiram Shkolnik, C. Richard Taylor*, Virginia Finch* & Arieh Borut

Department of Zoology, Tel Aviv University, Tel Aviv, Israel

* Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

Survival in hot deserts has always posed a problem for man; Moses had to solve it in order to lead the children of Israel through the wilderness of the Sinai—a formidable hot desert. It seems likely that the present inhabitants of the Sinai, the Bedouins, would have optimised their solutions for desert survival during their long tenure in this desert. Yet, one may have doubts on first encountering Bedouins wearing black robes and herding black goats. We have therefore investigated whether black robes help the Bedouins to minimise solar heat loads in a hot desert. This seemed possible because experiments have shown that white hair on cattle[1,2] and white feathers on pigeons[3] permit greater penetration of short-wave radiation to the skin than black. In fact, more heat flowed inward through white pigeon plumage than through black when both were exposed to simulated solar radiation at wind speeds greater than 3 m s^{-1} (ref. 3). We report here that the amount of heat gained by a Bedouin exposed to the hot desert is the same whether he wears a black or a white robe. The additional heat absorbed by the black robe was lost before it reached the skin.

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Table 1 Radiation exchange and convective heat loss

	Radiation gain	Radiation loss	Net radiation gain	Convective loss	Net radiation gain–convective loss
Black robe	838±10	543±2	295±8	57±7	238±12
White robe	649±3	534±3	115±2	31±5	84±6
Tan army uniform	644±3	452±2	192±2	45±3	147±4
Shorts (semi-nude)	624±10	442±6	182±5	−2±4	184±7

The rate of net heat gain by radiation was more than 2.5 times as great in the black robe as in the white. The greater heat loss by convection from the black robe due to its higher surface temperature was not great enough to compensate for its higher net radiation gain. This leads us to postulate that additional heat was lost by convection through the robes through a bellows effect or a chimney effect. Values in W m^{-2} are the means of nine experiments ± s.e.

To quantify the effects of the colour of a robe on the heat load imposed by a hot desert, we measured and/or calculated the net heat gain by radiation, heat loss by convection, heat loss by evaporation, heat storage and metabolic heat production of a man standing facing the Sun in the desert at mid-day while he wore: (1) a black Bedouin robe; (2) a similar robe that was white; (3) a tan army uniform; and (4) shorts (that is, he was semi-nude). We used one individual as a calibrated heat exchanger, because we were interested in the effects of the coverings rather than in physiological differences among individuals. Robes were purchased from Bedouins and we used two layers of robes in the same manner as they did. Only the outer robe was changed when comparing black and white robes. We selected experimental conditions where both solar radiation and air temperature were high. The measurements were made during the summer at mid-day (1030–1430 h) at the Hatzeva Field School (in the Negev Desert at the bottom of the rift valley between the Dead Sea and the Gulf of Elat). During the measurements air temperatures ranged between 35 °C and 46 °C (average 38 °C) and wind speed between 0 and 4.1 m s^{-1} (average 1.3 m s^{-1}).

Table 2 Avenues of heat gain and loss of a subject facing the Sun in a hot desert at mid-day and in a temperature-controlled room at 48 °C

	$\dot{H}_{\text{gain}}$	=	$\dot{H}_{\text{evap}}$	+	$\dot{H}_{\text{stor}}$	−	$\dot{H}_{\text{metab}}$	T_{amb}
Desert								
Black robe	142±4	=	199±4	+	10±1	−	67±2	59.5
White robe	134±11	=	191±8	+	8±1	−	65±2	58.6
Tan army uniform	161±7	=	217±7	+	10±1	−	66±2	–
Shorts (semi-nude)	208±7	=	264±8	+	8±1	−	64±2	62.2
Temperature-controlled room at 48 °C								
Black robe	66±7	=	126±6	+	7±2	−	67±2	
White robe	65±9	=	123±9	+	6±1	−	64±3	
Shorts (semi-nude)	86±9	=	144±9	+	9±2	−	67±2	

Rates of net heat gain ($\dot{H}_{\text{gain}}$) from the environment, evaporative heat loss ($\dot{H}_{\text{evap}}$), heat storage ($\dot{H}_{\text{stor}}$) and metabolic heat production ($\dot{H}_{\text{metab}}$) are given in W m^{-2} and are the means of nine experiments ± s.e. To determine the temperature in our environmental room (T_{amb}) that would be necessary to simulate conditions of our hot desert at mid-day, $\dot{H}_{\text{gain}}$ was quantified when the room was set approximately 10 °C above average skin temperature. It was then assumed that $\dot{H}_{\text{gain}}$ would be directly proportional to the temperature gradient between the skin and room in order to calculate the room temperature required to obtain the same $\dot{H}_{\text{gain}}$ as we obtained in the hot desert.

Radiation exchange and heat loss by convection were calculated according to the procedures outlined for cattle[4] and goats[5] with the following minor modifications. (1) Swinbank's empirical formula[6] was used for calculating long-wave radiation from the sky; (2) surface area was calculated from Dubois' formula[7] when the subject wore the army uniform or was semi-nude (this area was multiplied by 0.85 when calculating diffuse radiation exchanges to correct for exchanges between the legs, arms and body); and (3) surface area for the subject wearing the robes was calculated by assuming he approximated a cylinder whose circumference was measured around the chest.

Rates of metabolic heat production (H_{metab}), evaporative heat loss ($\dot{H}_{\text{evap}}$), and changes of heat content of the body ($\dot{H}_{\text{stor}}$) were measured, then net heat gain by non-evaporative means ($\dot{H}_{\text{gain}}$) was calculated by applying the first law of thermodynamics where:

$$\dot{H}_{\text{gain}} = \dot{H}_{\text{evap}} + \dot{H}_{\text{stor}} - \dot{H}_{\text{metab}} \qquad (1)$$

All measurements were made during 30-min intervals. The techniques and their accuracies were the same as we have described in detail for similar measurements on the Bedouin's black goats[5].

The absorptivity of the black robes for radiation in the visible part of the spectrum (0.89) was more than 2.5 times as great as that measured for the white robe (0.35) and about 1.5 times as great as for the army uniform (0.72) or the semi-nude subject (0.66). The greater rate of absorption of visible radiation by the black robes was not accompanied by a greater rate of radiation loss (Table 1). Although convective heat loss from the black robe was greater than that from the white because of its higher surface temperature, the net inward flow of heat through the black robe (that is, net radiation gain − convective loss) was calculated to be almost three times as great as through the white (Table 1).

In spite of the greater net heat gain by the black robe, there was essentially no difference in rates of net heat gain ($\dot{H}_{\text{gain}}$) by the subject wearing black or white robes (Table 2). Rates of evaporative heat loss, heat storage, and metabolic heat production were essentially the same in the black and white robes.

How does one reconcile these seemingly conflicting results? The explanation must be greater convective transfer of heat through the air spaces beneath the black robe, either by a bellows action as the robes flow in the wind or in a manner analogous to the functioning of a chimney where the chimney in this case would be the air space between the robes and the skin. The air beneath the robe might rise and pass through the loosely woven fabric as it was warmed, pulling in cooler air from the bottom of the robe. Either explanation would be supported by our measurments of temperatures in the air space between the robes and the skin. The temperature of the air space beneath the inner robe was approximately the same as the temperature of the surrounding air when the outer robe was black or white, despite as much as a 6 °C difference in surface temperature of the different coloured robes (47 °C for the black and 41 °C for the white) (Fig. 1).

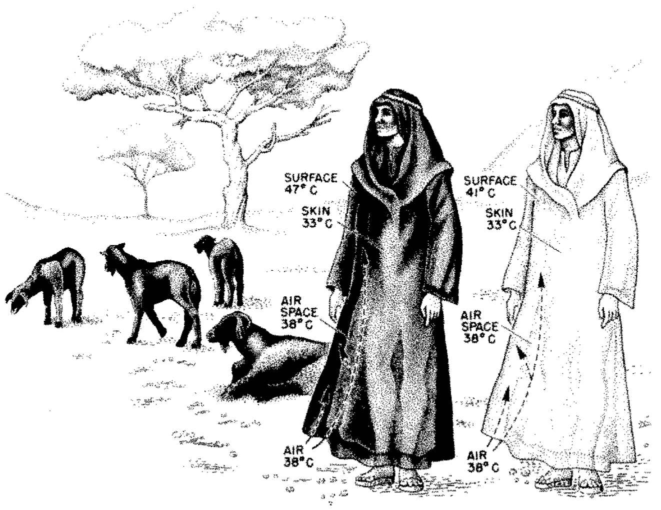

Fig. 1 Black Bedouin robes gain two to three times as much heat by radiation from the Sun as white robes, but enhanced convection of air beneath the robe carries this heat away before it reaches the skin. The temperature of the air space between the robe and the skin was the same for black and white robes and equalled the temperature of the surrounding air. The greater convection beneath the black robe might make it feel more comfortable. Mean temperatures of the robe surface, skin and air space (see text) are typical of temperatures observed when air temperature was 38 °C at mid-day, the sky was cloudless, and the subject was orientated facing the Sun.

Whether a bellows action, a chimney effect or some other mechanism explains the enhancement of convection beneath the black robes, it seems clear that the difference in radiation exchange at the outer surface of the robes is not affecting the heat exchange of the subject. Other authors have also observed that the colour of clothing has little effect on heat gain of a subject exposed to direct solar radiation[8,9].

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Data from kinships of monozygotic twins indicate maternal effects on verbal intelligence

Richard J. Rose[*], Joann A. Boughman[†], Linda A. Corey[†], Walter E. Nance[†], Joe C. Christian[‡] & Ke Won Kang[‡]

[*] Department of Psychology, Indiana University, Bloomington, Indiana 47405
[†] Department of Human Genetics, Medical College of Virginia, Richmond, Virginia 23298
[‡] Department of Medical Genetics, Indiana University School of Medicine, Indianapolis, Indiana 46233

Familial resemblance in intellectual skills is well documented, but its interpretation is a source of continuing controversy. The critical problem is that a family's shared genes are confounded with its shared experiences, and controls possible in animal research (selective mating, cross-fostering, and uniform or randomised environments) do not directly apply to human subjects. Conventional twin and family methods reveal substantial genetic variance in intelligence quotient (IQ) test scores, but the same methods also document significant environmental influences. Research designs which can identify the nature of these environmental factors may effect progress in the 'IQ debate' (ref. 1). The families of monozygotic (MZ) twins provide a new research design[2] which permits a unique assessment of maternal influences in quantitative traits. We describe here initial applications of the design to verbal IQ, with results suggesting that maternal effects significantly contribute to familial similarity in verbal intelligence.

Maternal effects influence cognitive development in early childhood[3]. Observations of mothers of adopted infants, which eliminate the confounding of genetic transmission with maternal attributes, have identified specific maternal behaviour which fosters intellectual development in infancy[4,5]. Characteristics of maternal care and qualities of the home environment during early life predict pre-adolescent IQ[6], and longitudinal studies in London[7] and Kauai[8] document effects of the quality and intensity of early maternal stimulation. These and other data[9–11] suggest that a mother's sensitivity to her infant's needs facilitates early intellectual growth. In part, cognitive developmental differences in childhood may reflect differences in children's interactions with their mothers.

Mothers are not merely models for imitation. They establish the conditions and incentives for children's early achievement. This pervasive, but indirect influence may significantly affect a child's cognitive growth without increasing mother–child resemblance, because the relevant attributes of a mother's child-rearing may be attitudes and expectations which are uncorrelated with her IQ. Thus, the fact[12] that children's IQs exhibit no greater resemblance to their mothers than to their fathers does not preclude significant maternal effects, as such effects can[13] and do[14] occur in the absence of increased mother–child covariance.

A direct test of maternal effects on intelligence is possible within the kinships of adult identical twins. Because their twin parents are monozygotes, the children of identical twins are related to one another as conventional half-siblings; socially, they are cousins reared in different households. Because they derive from twin parents, MZ half-sibships have the same expected age and size, in contrast to conventional half-sibships resulting from death, divorce or illegitimacy. The offspring of female MZ twins form a unique group of maternal half-siblings who allow a direct measure of maternal effects on human traits. When such effects are present, the offspring of female twins will resemble one another more than do the offspring of male twins.

Table 1 Analysis of variance of offspring data from two Wechsler verbal scales

Mean squares	Information		Vocabulary	
Among paternal (MS_{Ap})	8.56	$F' = 1.56, P < 0.05$	11.12	$F' = 1.37, P < 0.10$
Among maternal (MS_{Am})	13.13		16.03	
Between paternal (MS_{Bp})	5.53		5.69	
Between maternal (MS_{Bm})	3.66		4.77	
Within paternal	3.64	NS	3.91	NS
Within maternal	4.01		3.80	
Correlations				
Paternal half-siblings	0.125	$P < 0.05$	0.200	$P < 0.10$
Maternal half-siblings	0.334		0.328	

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For the Information subtest, the analysis is based on 335 offspring in kinships of 71 MZ twin parents ($N_p = 34$, $N_m = 37$). The analysis of Vocabulary data is based on 397 offspring in 79 MZ kinships ($N_p = 37$, $N_m = 42$). Because the number of offspring within each sibship varies, coefficients of the between, within and among components of variation are calculated from observed distributions of family size[13,14]. The three coefficients do not differ for paternal and maternal families in the present data, permitting a direct evaluation of maternal influences by an F' test

$$F' = MS_{\mathrm{Am}} + MS_{\mathrm{Bp}}/MS_{\mathrm{Bm}} + MS_{\mathrm{Ap}}$$

$$\text{with d.f.} = \frac{(MS_{\mathrm{Am}} + MS_{\mathrm{Bp}})^2}{(MS_{\mathrm{Am}})^2/N_m - 1 + (MS_{\mathrm{Bp}})^2/N_p} \text{ for numerator and d.f.} = \frac{(MS_{\mathrm{Bm}} + MS_{\mathrm{Ap}})^2}{(MS_{\mathrm{Bm}})^2/N_m + (MS_{\mathrm{Ap}})^2/N_p - 1} \text{ for denominator}$$

In controlled matings of laboratory animals, each of a group of sires is mated to multiple dams, and the resulting litters of half- and full-sibling progeny permit a direct evaluation of maternal effects through a nested analysis of variance[15]. Figure 1 shows that the offspring of identical twins provide a human parallel to these conventional animal methods. Variation among the offspring of MZ twin parents may be partitioned by a nested analysis of variance[16] into components arising (1) among families, (2) between sibships within families, and (3) within sibships. Because the offspring of male twins are born to genetically unrelated mothers, maternal effects, when present, will inflate the variation arising between sibships within paternal families (MS_{Bp}). Conversely, maternal effects will inflate the variance arising among half-sibships in maternal families (MS_{Am}). Finally, because all offspring within each sibship are born to the same mother, maternal effects will not affect variation arising within sibships. Accordingly, a direct test of maternal effects can be made from the partitioned variance of offspring data.

To evaluate maternal effects in intellectual development, we administered the vocabulary and information subtests from the Wechsler intelligence scales to offspring of MZ twins. Of the 11 Wechsler subtests, Vocabulary and information are the most reliable, and factor analyses of the Wechsler scales suggest that Information and Vocabulary are consistently the best predictors of general intellectual functioning over the entire age range. In addition, because the focus of this report is on maternal effects, these two verbal scales are of special interest given evidence of the mother's dominant role in a child's early development of verbal skill[17].

Subjects were tested individually by trained examiners. Offspring of ages 4.0–5.11 yr were administered the Wechsler pre-school and primary scales; those aged 6.0–15.11 yr, the 1975 revision of the Intelligence Scales for Children, and those over age 16 yr were administered the Wechsler adult intelligence scale. For time intervals up to 1 yr, the inter-test correlations for vocabulary and information are as high as their test–retest stability on the individual Wechsler scales[18,19]. Some self-selection, expected in voluntary research, is evident in our study population and verbal scores of the sample reveal elevated means and restricted variances compared with national norms. However, means and variances do not differ between twins and non-twins, males and females, or children and adults; the family providers in maternal and paternal families are closely matched in educational attainment and social-class position and comparisons between their offspring are not invalid.

Results of the analysis of variance of offspring data are presented in Table 1. The analysis provides direct evidence of a maternal effect on verbal intelligence. For the information subtest, the intra-class correlation of maternal half-siblings, offspring of MZ twin mothers, significantly exceeds that of paternal half-siblings; comparison of the mean squares yields $F' = 1.56_{61,56}$, which enables us to reject ($P < 0.05$) the null hypothesis of no maternal influence. Results for the vocabulary subtest are similar, although the F' test does not achieve statistical significance in this preliminary sample.

The maternal effect suggested in these results could arise in any of several ways, some unique to kinships of twin mothers, some not. (1) Twin sisters may spend more time together during childhood than do twin brothers, and as a consequence of more similar childhood experience, may be more alike in their tested intelligence. We evaluated that possibility by comparing the IQ correlations of our male and female twin parents. No difference was found. (2) Female twins may maintain more frequent social contact in post-marital life and create more overlap in the IQ-relevant experience of their children. An adequate test of this possibility awaits collection of self-reported frequency of social interaction within kinships, but a crude test was made by comparing the geographical propinquity of maternal and paternal half-sibships. Again, no difference was found. (3) Assortative mating for IQ by MZ twin sisters may exceed that by MZ twin brothers. Asymmetric assortative mating was directly evaluated by comparing the resemblance of wives of twin brothers to husbands of twin sisters. The spousal correlations do not differ. (4) Identical twin sisters may provide more similar patterns of child-rearing than do the unrelated wives of twin brothers, such that the greater resemblance of their children reflects greater resemblance of relevant maternal behaviour. (5) The greater resemblance of maternal half-siblings may reflect

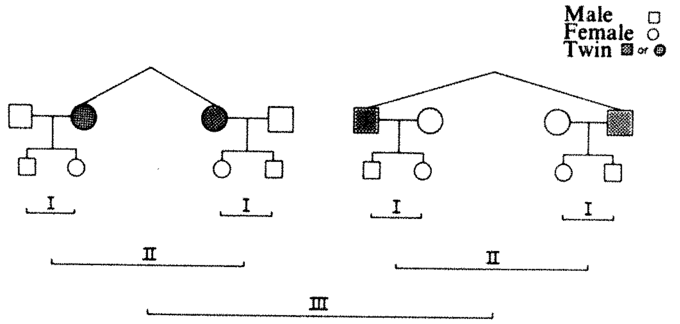

Fig. 1 Variation in offspring data in kinships of MZ twin parents. I, A component of variation arising within sibships; II, variation arising between sibships nested within families; III, variation arising among families.

the reduced variability of intra-uterine environment with which identical twin mothers provide their children. The last two possibilities reflect pre- and postnatal maternal influences not limited to kinships of twin parents, and, although we have no direct tests of them, research with mothers of singletons suggests their plausibility.

Maternal effects modulate the expression of familial mental retardation[20], and the data presented here, obtained from normal children reared by their biological parents in typical home environments, provide direct evidence of significant maternal influences on variation in tested IQ. Indeed, the correlation of maternal half-siblings approaches that typically reported for full siblings! We remain uncertain as to whether the source of these maternal influences is pre- or postnatal, but we emphasise that they are found in the context of significant genetic variance[21] to clarify a widespread confusion—that demonstrations of genetic variance in IQ test scores nullify social efforts to enrich early intellectual development. Heritability of IQ does not negate its social modifiability. Evidence of significant maternal effects may serve to redirect the polemical IQ debate towards constructive efforts to identify environmental factors in cognitive development.

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Distracting information, motor performance and sex differences

Judith I. Laszlo, Phillip J. Bairstow, Gary R. Ward & Hudson Bancroft

Departments of Psychology and Physiology, University of Western Australia, Nedlands, Western Australia 6009

It is often argued that, in general, men are superior to women in perceptual motor skills (for example, car driving). Extensive reviews of the relevant literature do not give convincing evidence of sex differences in manual dexterity[1] or motor skills in general[2]. However, in the studies reported here, variables have been isolated which demonstrate that sex differences do exist in two perceptual motor tasks. These studies showed that females are adversely affected by irrelevant stimuli while performing tasks permitting freedom in movements but perform as well as males when the movement is stereotyped.

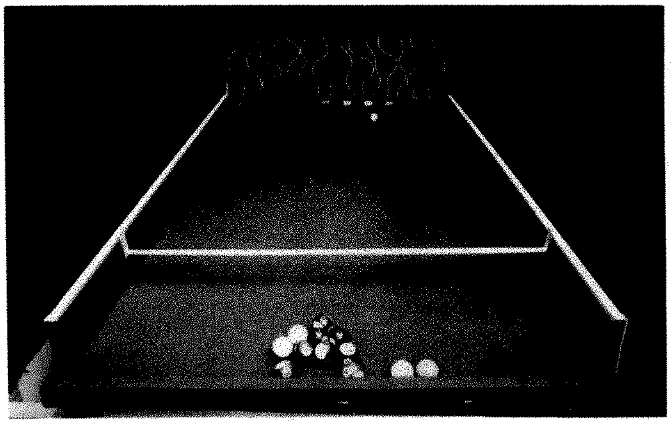

Fig. 1 The experimental set-up. The subject was to roll the ball at the target aiming at the centre flap. In Ward-games *a* and *b* the target comprised seven flaps—the centre flap scored 4; in Ward-game *c* nine flaps were used, the centre flaps scored 5. The landmarks shown were used in Ward-game *c*. In the no-landmark condition the lines were absent.

We first established the performance variables that have direct relevance to motor programming in an aiming task, without considering sex differences. The task was carried out on a Ward table 213 × 114.5 cm (see Fig. 1). At one end was a target consisting of seven 5-cm-wide flaps, either stationary in the middle of the table, or moving from side to side at 20 excursions per min. Subjects had to hit the target with a billiard ball. The score depended on the accuracy of the hit. Target missed scored 0; the outer two flaps scored 1 each; the middle flap scored 4, with increasing scores for the second and third flaps. Several variables were tested in a between-subjects, randomised block design, but for the present purposes, only motor programming demands and sensory information are considered. Motor programming demands varied from simple stereotyped to complex movements, involving temporal and/or spatial organisation. (1) The subject was required to 'shoot' the ball out of a spring-loaded 'gun' at the moving target. The subject had to make the temporal decision when to press the button to release the ball. (2) The subject rolled the ball manually at the stationary target, requiring a directional decision only. (3) The ball was rolled manually at the moving target, necessitating both spatial and temporal programming.

Sensory information was varied by the presence or absence of landmarks. Above the target was a matt blackboard in the no-landmark condition, and four regularly placed vertical white lines were displayed on the blackboard in the landmark

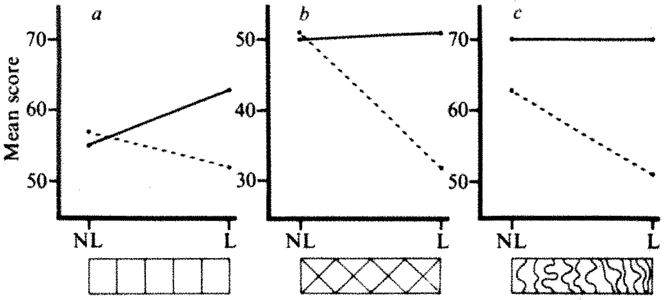

Fig. 2 Results of three Ward-games with the ball manually rolled at the moving target. Maximum score for: Ward-game *a* [condition (3)], 30 trials = 120; Ward-game *b*, 25 trials = 100; Ward-game *c*, 25 trials = 125. Males: ——; females: - - -. L, landmark; NL, no landmark. The patterns below each graph denote the visual stimuli used as landmarks.

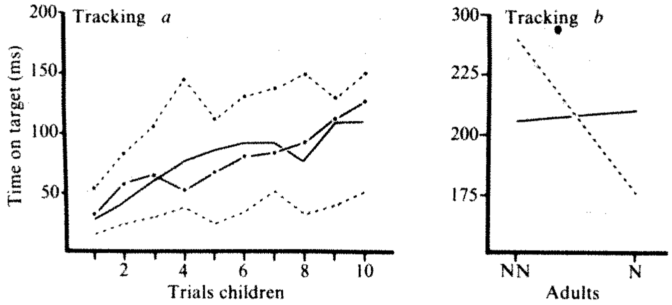

Fig. 3 Results of tracking studies in adults and children. Males: ——; females: - - -. Tracking *a*, noise: no dots; no noise: dots marking data points. Tracking *b*, N: traffic noise through earphones; NN: no noise.

condition. These landmarks were expected to aid in the timing of the movement.

The experiment was conducted as the 'Ward-game' championship and we noticed that males dominated the finals. The results showed that in none of the movement conditions did the landmarks assist male performance, whereas females performed significantly worse with landmarks than without landmarks in conditions (2) and (3) [(2) $t(32) = 3.52$, and (3) 3.92, $P < 0.01$], although in (1) no significant difference was found. Thus, landmarks affected females only, and were irrelevant for the performance of males (Fig. 2).

We carried out two further experiments with the subjects manually rolling the ball at the moving target [condition (3) Ward-game *a*], but varied the visual patterns (Fig. 2). The results confirmed the findings of Ward-game *a*. [Ward-game *b*: landmark main effect $F(1, 36)$ 5.102, $P < 0.03$; sex main effect $F(1, 36)$ 7.662, $P < 0.01$; interaction 9.072, $P < 0.005$; Ward-game *c*: with planned comparison: males versus females with landmark $F(1, 36)$ 9.84, $P < 0.01$; males versus females without landmarks $F(1, 36)$ 0.28, not significant.]

To establish that sex differences were not task specific and relevant to visual stimuli only, two pursuit tracking studies were run. In these, a triangular track (33 r.p.m.) was used, with recorded traffic noise as the irrelevant stimulus. For the control groups the noise was omitted. In the tracking *a* study, the subjects were 7–12-yr-old children, and in tracking *b*, adults were used. The Ward-game results were confirmed [tracking *a*: auditory main effect $F(1, 28)$ 6.96, $P < 0.01$; sex main effect not significant; interaction 5.81, $P < 0.01$; tracking *b*: ANOVA results not significant, *t*-test on female performance with versus without auditory noise: $t(22) = 4.214$, $P < 0.001$] (see Fig. 3).

In a further three experiments with stereotyped aiming movements[3] and visual distractors, no sex difference was found.

Thus, it seems that, as a group, females from the age of 7 yr are adversely affected by irrelevant stimuli in the performance of motor skills in which spatial freedom of movement programming is possible. It can be argued that the input which is processed preceding and determining the motor programme[4] is over-inclusive in females, but selective in males.

The implications of these findings are: (1) contradictory results in the literature on sex differences in motor skill may be due to stimulus configuration in the experimental situation and/or to motor programming demands of the task; (2) most females might be adversely affected by distracting stimuli when operating complex machinery and in car driving; (3) industrial training and driver education may need to be re-examined.

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Origin of the mammal-like reptiles

T. S. Kemp

University Museum, Parks Road, Oxford, UK

The first major adaptive radiation of reptiles consisted of the Synapsida, or mammal-like reptiles, which are now totally extinct, but which dominated the terrestrial fauna from the late Pennsylvanian, throughout the Permian and for much of the Triassic periods. The mammals arose from advanced synapsids in the Upper Triassic, at which time the dinosaur reptiles were rapidly replacing the mammal-like reptiles as the principal terrestrial forms. The orthodox view[1,2] of the ancestry of this important stage in vertebrate history is that the synapsids arose from a hypothetical stem-reptile of the family Romeriidae, the group closest to the ancestry of all the other reptile lineages. The principal difference between romeriids and the primitive synapsids is the development of a space, or temporal fenestra in the cheek region of the skull of the latter. However, the structure of this part of the skull of the romeriids cannot satisfactorily be considered ancestral to the synapsid temporal region on either comparative anatomical or functional grounds. As I propose here, the kind of skull which would be expected to evolve into that of a synapsid is the type possessed by the archaic 'quasi-reptile' *Limnoscelis* and its possible relative *Romeriscus*.

The typical synapsid temporal fenestra is already fully developed in the earliest known skull[3], that of the pelycosaur *Archaeothyris* from deposits of Westphalian D age, in the Pennsylvanian of Nova Scotia (Fig. 1*b*). The fenestra is bounded above by the postorbital and squamosal bones, and the parietal bone is not in contact with the squamosal. Another point to note is that the supratemporal bone, although small, still contacts the postorbital. In contrast, the romeriid skull (Fig. 1*a*) has the postorbital restricted anteriorly, so that there is a long line of contact between the parietal and squamosal, while the supratemporal is reduced to a small, superficial bone right at the back of the skull. The only explanation offered[4] for the difference in bone configuration is that when the temporal fenestra formed in a romeriid-like skull, there was a secondary extension of the postorbital bone backwards, above the new fenestra, to strengthen this part of the skull. It is not clear, however, why the temporal region of the skull should be strengthened in this way. If fenestration of a romeriid skull were to occur, then the space would inevitably develop between the parietal bone above and the squamosal bone below, yet these are two of the largest, most robust bones of the skull, and their mutual contact both in front of and behind the supposed new fenestra would have provided adequate strength. Indeed, this is probably what happened in certain other reptile lines where the fenestra is bounded dorsally by the parietal and squamosal. An alternative view of the origin of the synapsid skull is that the ancestral condition of the temporal region consisted of a large supratemporal bone which was in contact anteriorly with the postorbital. This condition is found in the skull of the limnoscelids, which are early reptile-like forms best known as *Limnoscelis* itself[5]. *Limnoscelis* occurs in the Lower Permian of North America and is a relatively large animal. The skull is unique among early reptiles, for the supratemporal bone is very large and extends far enough forwards to make a broad contact with the postorbital (Fig. 1*c*). The supposed remnant[6] of the old crossopterygian fish hinge-line

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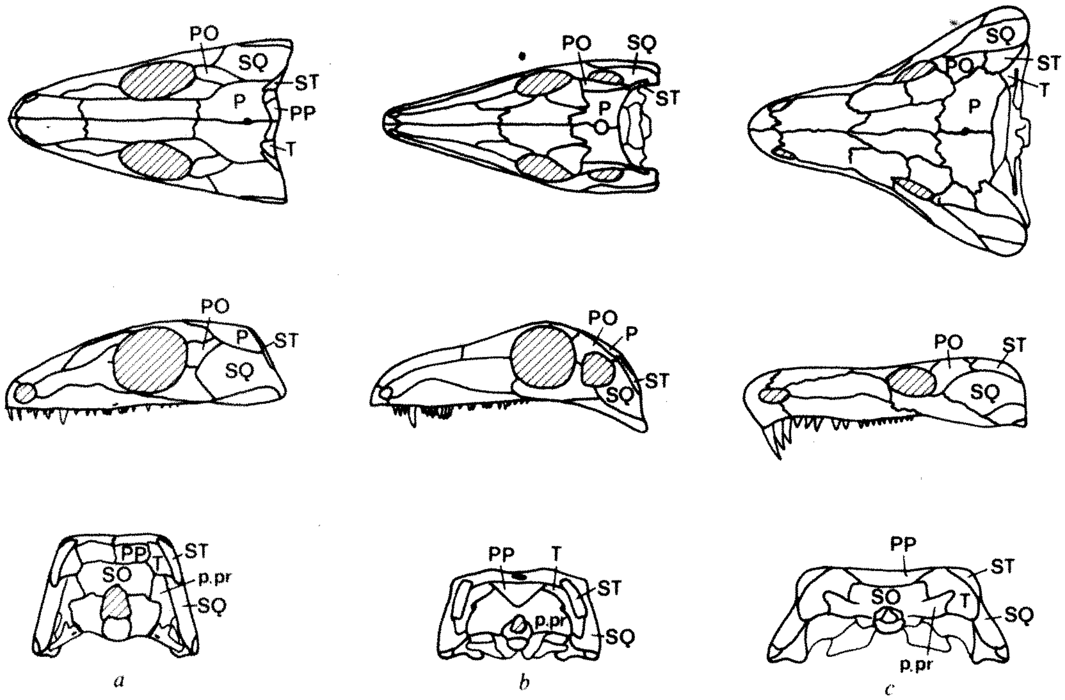

Fig. 1 *a*, Skull of the primitive romeriid *Paleothyris* in dorsal (top), lateral (centre) and posterior (bottom) views. Note the extensive contact between the parietal (P) and squamosal (SQ), the short postorbital (PO) and small supratemporal (ST). No temporal fenestra is present (after Carroll and Baird). *b*, Skull of pelycosaur synapsids, *Archaeothyris* in dorsal view (top) and lateral view (centre); *Varanops* in posterior view (bottom). Note the contact between the postorbital (PO) and supratemporal (ST) and postorbital and squamosal (SQ) above the temporal fenestra (after Romer and Price, and Reisz). *c*, Skull of the primitive reptile-like *Limnoscelis* in dorsal (top), lateral (centre) and posterior (bottom) views. Note the large contact between the postorbital (PO) and supratemporal (ST) bones above the line of weakness between these two bones and the squamosal (SQ) (after Romer). m, adductor mandibuli muscle; P, parietal; PO, postorbital; PP, postparietal; p.pr, paroccipital process; SO, supraoccipital; SQ, squamosal; ST, supratemporal; T, tabular; t.ap, temporal aponeurosis.

persists in the form of a failure of the squamosal to unite firmly with the supratemporal and postorbital above. Thus the cheek and skull table must have been held together by soft connective tissue, probably to allow a slight intracranial kinesis at this point. Another, much earlier form *Romeriscus* has been described as a limnoscelid by Baird and Carroll[7] (Fig. 2*a*), on the basis of a similar configuration of the temporal region. In fact, the skull is very incomplete and badly crushed, but certainly the supratemporal is large, and extends anteriorly sufficiently for it to be presumed to meet the missing postorbital. And there is no apparent suture between the supratemporal and squamosal, indicating the presence of a line of weakness. Whether *Romeriscus* is a limnoscelid or not is doubtful[6], but it does suggest that the basically limnoscelid type of temporal region of the skull existed early, for it dates from the Westphalian A, earlier than any of the true reptiles.

The arrangement of the skull bones of *Limnoscelis* offers a good model for the ancestral state leading to the fenestrated synapsid condition. The classic view that a temporal fenestra is a window out of which the contracting jaw muscles can bulge has been discredited by the observation that in modern fenestrated reptiles the muscles do not so bulge, and that the muscle fibres tend to attach to the edges of the fenestra and to connective tissue covering it. A more acceptable view is that of Frazzetta[8], who suggested that the role of the fenestra is to provide bony edges, to which the muscles can be more strongly anchored. In the line leading to pelycosaurs, an increase in body size appears to have occurred, for all the early forms are larger than other contemporary reptiles[3]. Therefore a relative increase in the mass of the adductor jaw musculature would be expected. If the ancestral, unfenestrated skull was similar to that of limnoscelids, some of the muscle fibres would tend to become attached to the connective tissue between the skull table and cheek (Fig. 2*b*, top). In effect, these particular fibres would be attached to the lateral edge of the postorbital and supratemporal bones, giving them a stronger anchorage than other fibres which merely attached to the internal surfaces of the skull bones. An increase in the area of the connective tissue between cheek and table so that increasing numbers of muscle fibres could attach to it would be expected, leading to the development of a gap, or fenestra, between the postorbital and supratemporal above and the squamosal below (Fig. 2*b*, bottom), covered over by an aponeurotic sheet. The weakest point of the temporal region would have been the supratemporal bone, for it is small and not strongly attached posteriorly. Expansion of the postorbital posteriorly, below the supratemporal, to meet and brace against the squamosal followed, thereby strengthening the post-fenestral region of the skull, and giving rise to the characteristic form of the synapsid postorbital bone.

Two further arguments support this hypothesis of the origin of the synapsid skull. The first concerns the occipital structure of the skull. In the romeriids, the tabular bone is small and restricted to the dorsal part of the occiput, and therefore makes at best a very limited contact with the paroccipital process (Fig. 1*a*, bottom). In contrast, the pelycosaurs possess a large tabular which extends ventrally to make a massive, bracing contact with the paroccipital process (Fig. 1*b*, bottom). Panchen noted this point[6], and concluded that the common ancestor of romeriids and pelycosaurs was more primitive than any known form. In fact, the limnoscelid tabular and paroccipital process have a relationship very similar to that of the pelycosaurs (Fig. 1*c*, bottom). Functionally, retention of the tabular-paroccipital process brace in pelycosaurs would be expected, as a further manifestation of the need for strengthening devices in the potentially weak post-fenestral region of the skull. The second argument is that in certain generally primitive pelycosaurs such as *Eothyris*[1] (Fig. 2*c*) and *Oedaleops*[9] the supratemporal is a large bone, making extensive contact with the postorbital directly above the temporal fenestra. It is not unreasonable therefore to suggest that a large supratemporal bone, quite unlike that of the romeriids, is actually the primitive synapsid condition.

The conclusion is, therefore, that the synapsid skull evolved from a limnoscelid-like skull rather than from that of a romeriid, and that the temporal fenestra of the mammal-like reptiles evolved directly from the remnant of the crossopterygian hinge line. This is not to say, of course, that taxonomically the limnoscelids are the ancestors of the synapsids, merely that the limnoscelids have retained the ancestral configuration of the temporal region of the skull. It has long been supposed that a fundamental reptilian feature is the parietal lappet, which is the lateral process of that bone which contacts the squamosal and replaces topologically the intertemporal bone of early labyrinthodont amphibians. This arrangement contrasts with those labyrinthodonts which themselves lost the intertemporal, for here the area occupied by that bone is taken over by the postorbital and supratemporal. On the argument presented here, the synapsid line at least never possessed a parietal lappet. Whether the parietal lappet of the romeriids and other primitive reptiles does indeed indicate a separate loss of the intertemporal, or whether it reflects a subsequent reduction of the postorbital and supratemporal bones is not known. The latter seems the more probable in view of the many points of similarity that do exist between limnoscelids, romeriids and pelycosaurs.

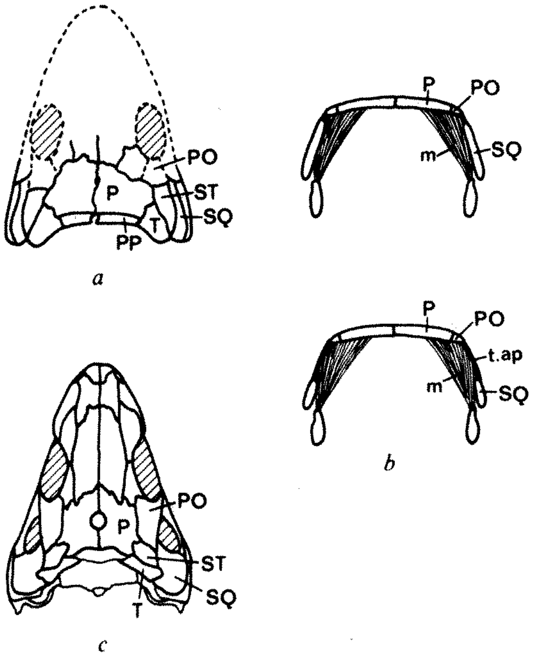

Fig. 2 *a*, Skull of the possible early limnoscelid *Romeriscus* in dorsal view (after Baird and Carroll). *b*, Above, diagrammatic cross-section through the temporal region of the skull of a limnoscelid-like form, in which the adductor jaw muscles (m) are beginning to gain an attachment to the connective tissue binding the postorbital (PO) of the skull roof to the squamosal (SQ) of the cheek. Below, further development of this trend, with enlargement of the gap to form a temporal fenestra, covered by a large connective tissue aponeurotic sheet (t.ap), leading to the synapsid skull pattern. *c*, Dorsal view of the skull of a very primitive pelycosaur synapsid *Eothyris*. Note the retention of a large supratemporal in contact with the postorbital (after Romer and Price).

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Juvenile ceratopsians from Mongolia—the smallest known dinosaur specimens

Walter P. Coombs Jr

Department of Biology, Western New England College, Springfield, Massachusetts 01119

Among dinosaurs collected by the Third Asiatic Expedition of the American Museum of Natural History (New York) are two juveniles each of which is smaller than any hitherto reported dinosaur specimen. Their overall anatomy resembles that of the type specimen of *Psittacosaurus mongoliensis* (AMNH 6254)[1], which was collected from the same horizon and locality: the Lower Cretaceous Oshih Formation, Artsa Bogdo Basin, Mongolian Peoples' Republic. The anatomy of the new specimens confirms *Psittacosaurus* as a ceratopsian, not an ornithopod, and their minute size precludes both post-hatching parental care and endothermy.

Of the two specimens, AMNH 6535 includes a skull and partial mandibles and AMNH 6536 includes a skull, mandibles, partial pes in articulation and numerous postcranial elements (Fig. 1). Important cranial features include: skull triangular in dorsal view; short deep snout with parrot-like beak; small nostrils far dorsal; massive jugal with small ventrolateral flange

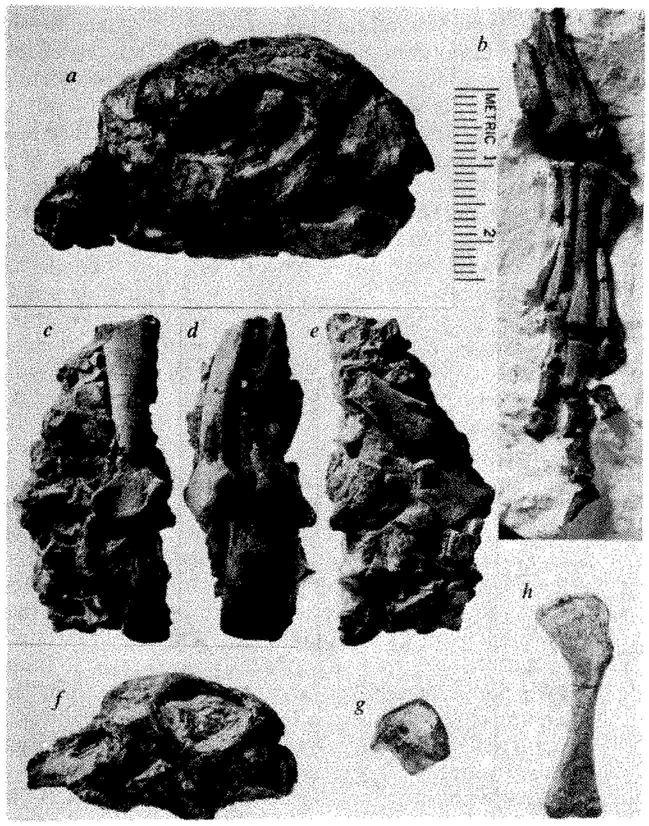

Fig. 1 Representative material of the two smallest known dinosaur specimens. *a–e*, *g* and *h*, AMNH 6536; *f*, AMNH 6535. *a*, Skull and mandibles. *b*, Left pes with distal ends of tibia and fibula, astragalus, calcaneum, metatarsals II, III, IV, and partial metatarsal V, and a partial set of phalanges. *c*, *d*, And *e*, three views of a block of bone containing two scapulae, partial ilium, partial ischium, proximal end of a femur, several vertebrae, and sacral ribs. *f*, Skull and partial mandible. *g*, Right coracoid. *h*, Left humerus. Scale divisions in mm (shown approximately natural size).

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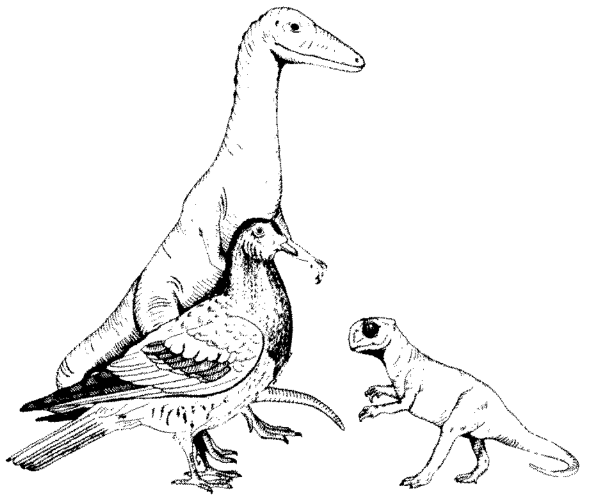

Fig. 2 Restoration of the smallest known dinosaur, a juvenile *Psittacosaurus mongoliensis*, based on AMNH 6535. Included for size comparison (on the left) are *Compsognathus longipes* and a common pigeon (*Columba livia*). Illustration by Matthew Hyman.

or spike; and massive curved mandible with triangular coronoid process. A rostral bone, present on both juvenile skulls and the type specimen of *Psittacosaurus mongoliensis*, is a synapomorphic character diagnostic of the suborder Ceratopsia. Wear on the teeth of both juvenile skulls indicates a diet of abrasive vegetation, and suggests that a newly hatched *Psittacosaurus* would have been smaller than either of these specimens. Pedal conformation, humeral shape, elongate tapering scapulae and femora with cylindrical heads and curved shafts (Fig. 1) all indicate a gracile, tridactyl biped. That these specimens are juvenile is indicated by the enormous orbits, relatively large braincase, arched cranial roof, short snout and neural arches not fused to centra. Using basicranial length for comparison, the smallest known dinosaur specimens include the following: *Lesothosaurus diagnosticus*, 94 mm; two juvenile *Protoceratops andrewsi*, 76 mm and 62 mm; *Compsognathus longipes*, 70–75 mm, and *Bagaceratops rozhdesvenskyi*, 47 mm (refs. 2–6). The juvenile *Psittacosaurus* skulls measure 42 mm (AMNH 6536) and 28mm (AMNH 6535). Estimated total body length, snout to tail tip, obtained by scaling down the complete skeleton of *P. mongoliensis* (AMNH 6254) is 395 mm for AMNH 6536, and 265 mm for AMNH 6535. These values make no allowance for allometric changes in proportions, and insofar as the skull is relatively larger in juveniles, the actual total length for AMNH 6535 is probably between 230 and 250 mm. The latter juvenile was smaller than a common pigeon (*Columba livia*) and less than one-third the length of *Compsognathus longipes* (750–810 mm)[6], also a juvenile specimen and often cited as the smallest known dinosaur (Fig. 2).

Dinosaur egg morphology indicates a pattern of nest construction similar to that of crocodilians and megapodes (chicken-like, terrestial birds of Australasia)[7], and parental attendance of such nests is reasonably inferred for dinosaurs. The usual thickness of dinosaur eggs may have reduced breakage by attendant parents of large size and fully upright stance. However, the minute size of these juvenile *Psittacosaurus* compared with the adult (body mass of juvenile about 0.7% of adult mass) makes post-hatching care by parents virtually impossible, both because the parent could not effectively feed so tiny a juvenile, and because the young would be liable to crushing at the least misstep of the adult. Tooth wear and well-ossified limb bones point to precocial self-feeding by hatchling *Psittacosaurus*. The surface area to body mass ratio of these juveniles would promote heat loss at a much higher rate than in adults. Exceptionally small neonates of modern endotherms are commonly ectothermic at birth, with endothermic physiology developing together with a more favourable surface area to body mass relationship[8,9]. Egg volumes of *Protoceratops* and *Hypselosaurus* infer a hatchling to adult size discrepancy even greater than that of *Psittacosaurus*[10]. Regardless of adult physiology, it is unlikely that such relatively tiny juvenile dinosaurs were endothermic in a mammalian-avian sense.

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Male and female visual neurones in dipterous insects

N. J. Strausfeld

European Molecular Biology Laboratory, Postfach 102209,
6900 Heidelberg, FRG

In many insects some visually guided behaviour patterns differ between the sexes. For example, male hoverflies and houseflies chase females in the air using visual cues but are not chased by females[1–3]. Presumably information processing by nerve cells also differs. The genetic and histochemical identification of a brain area in *Drosophila* that must be male for male behaviour to occur[4] suggests that circuits for sexual behaviour are localised. Recent anatomical studies of houseflies (*Musca domestica*) have demonstrated certain visual neurones present only in males[5]. I report here results from another species (the blowfly, *Calliphora erythrocephala*) demonstrating another kind of sexual difference of neural architecture in the visual system. In this fly dimorphism is expressed by differences in the shapes of analogous neurones in males and females, as well as by the presence of some cells in only one sex. The sexually dimorphic neurones of both sexes 'view' a region of the visual field coinciding with the region of binocular overlap.

The structures described here are from the lobula, the third visual neuropil. In this neuropil, columnar arrangements of small neurones precisely represent the facet organisation of the retina, and any dendritic field in the lobula can be represented as a map on the retinal surface[5,6]. Assemblies of nerve cells in the lobula are revealed by silver intensification of cobalt ions introduced either from a pool of cobalt chloride over a cut in the thoracic ganglion or from a smaller pool diffusing from a micropipette inserted into the brain. In the first case cobalt ions pass 'trans-synaptically' from dendrites of descending neurones into presynaptic relay cells originating in the lobula (Fig. 1*a*, *b*)[7]. In the second case cobalt appears to be taken up directly by one or more populations of visual interneurones[8]. The same neuronal arrangement can be revealed reproducibly in different brains. The results reported here are obtained from examination of 80 males and 60 females.

The shapes of many neurones do not significantly differ in males and females and comprise similarly structured networks. These are not described here; instead the emphasis is on morphologies characteristic of one or the other sex.

Lobulae of male blowflies have certain prominent neurones, each with characteristic shape, dendritic domain and axonal destination. Three of these are the male lobula giants[5] (MLG 1–3) illustrated in Figs 1 and 2. Two (MLG 1, 2) reside in the upper frontal lobula where their unistratified dendritic fields interact with relays from upper frontal ommatidia of the eye

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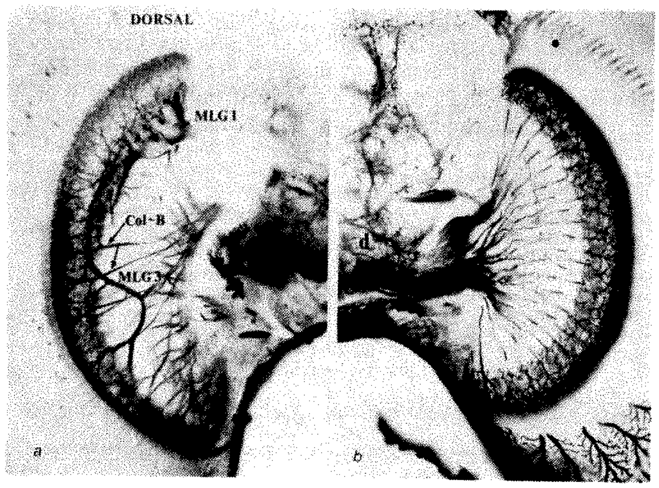

Fig. 1 Silver intensified neurones in male (*a*) and female (*b*) lobulae of *Calliphora erythrocephala*. The two pictures illustrate palisades of Col A neurones, parts of two male lobula giant neurones (MLG 1 and 3), and some Col B neurones which have taken up cobalt after its initial introduction into cut thoracic ganglia (×100).

(Fig. 3). Their cell bodies lie as a pair posteriorly above the upper margin of the lobula plate. The axon of MLG 1 passes to the rear of the contralateral mid-brain, ending among dendrites of descending neurones. The axon of MLG 2 ends at the equivalent ipsilateral region. Terminal branching patterns of MLG 1 and MLG 2 are similar. The third giant neurone (MLG 3) has a lyre-shaped profile. Its dendritic branches invade the anterior lobula along a strip that is narrow dorsally and wider ventrally (Fig. 3). The location of its cell body has not been identified. Its axon projects to the vicinity of a descending neurone in the medial ipsilateral protocerebrum.

In the female lobula there are four special neurones (female lobula giants; FLG 1-4). The dendrites of two of these (FLG 1, 2) invade the upper frontal lobula. FLG 1 is characterised by a unistratified field of spiny dendrites and, like MLG 1, its axon ends among dendrites of descending neurones in the rear of the contralateral mid-brain. The dendrites of FLG 2 are multistratified and distal to FLG 1 in the lobula. Like MLG 2, its axon passes to the rear of the ipsilateral mid-brain. The cell bodies of FLG 1 and 2 lie as a pair between the upper margin of the lobula and the upper margin of the lobula plate. A small lyre-shaped neurone (FLG 3) invades a narrow anterior strip of the lobula both above and beneath its equator. Like MLG 3, its axon projects ipsilaterally to the medial protocerebrum. FLG 4 invades a fronto-ventral strip of the lobula. Its axon projection has not been followed and the cell body locations of FLG 3 and 4 are not known.

Sexual dimorphism is also expressed among palisades of columnar neurones common to both sexes. Type A columnar cells (Col A)[5] are distributed throughout male and female lobulae and their axons project to the same region of the medial ipsilateral protocerebrum as MLG 3 and FLG 3. Col A shapes and distributions are similar in the two sexes (Fig. 2). However, in the upper region of the male lobula, where they overlie MLG 1 and 2, Col A cells show a graded change of dendritic morphology from multistratified to bistratified. In females, Col A neurones are uniform over the whole lobula, including the region occupied by FLG 1 and 2.

Both sexes of blowfly have in the lobula an upper-frontal row of columnar neurones serving exclusively the extreme frontal retina. In males, this row consists of two cell types: Col B ventrally, and Col C dorsally. In females the row consists only of Col B cells, ventrally and dorsally (Fig. 2). Col B cells have multistratified dendrites and long oval dendritic domains. Their axons project dorsally to ipsilateral protocerebrum in both sexes. Col C cells are distinguished by flattened dendritic expansions emitting numerous thin processes. Col C axons

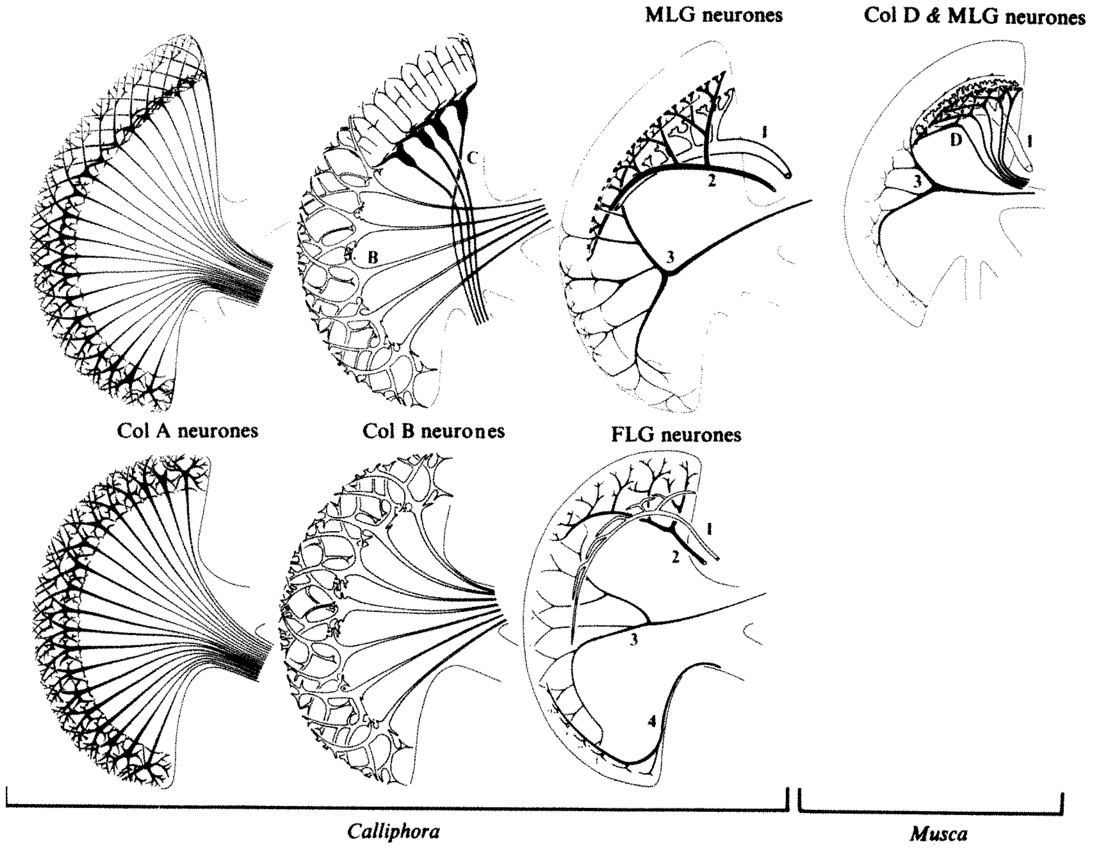

Fig. 2 Diagram of neurones in the lobula of male blowflies (upper row) and females (lower row) as seen in vertical sections of the lobula. Dorsal is to the top, with the midline to the right. Type A columnar neurones (Col A) are found throughout the lobula and are identical between the sexes except in the upper frontal zone of the male lobula. Col B neurones are found in the anterior of the lobula in both sexes. However, in males they are absent from an upper region. Col C neurones are found only in the anterior upper male lobula. Three unique male lobula giant neurones (MLG 1-3) and four unique female lobula giant neurones (FLG 1-4) are also found. Although termed female giants the diameters and processes of FLG neurones are usually much smaller than those of MLG neurones. The inset (upper right) shows the homologues of the MLG 1-3 neurones in the housefly, where a palisade of columnar cells (Col D) appears to substitute the single MLG 2 of *Calliphora* (see ref. 5).

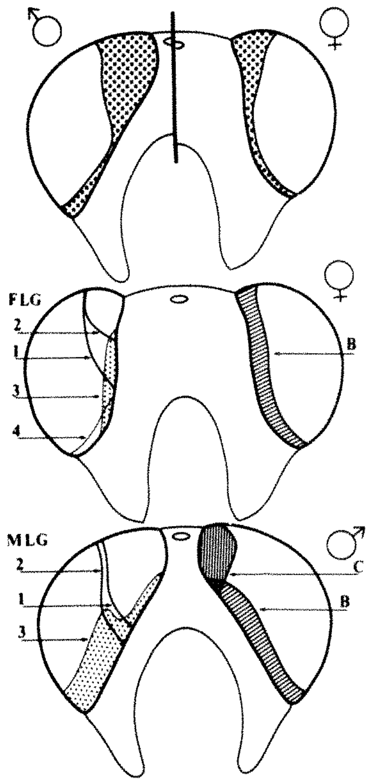

Fig. 3 Maps of dendritic fields demonstrate the sexual dimorphism. The top figure juxtaposes front views of male and female half-heads to show differences in eye position and zones of binocular overlap[9] (indicated by heavy dots). The middle figure shows the domains of FLG neurones mapped on to the retina to the left. The domains of FLG 1 and FLG 2 are above the lines indicated; the domain of FLG 3 is dotted and the domain of FLG 4 is below the line indicated. The domain of female Col B cells is shown in hatching on the right. The lower figure illustrates the domains of MLG 1-3 and male Col B and Col C neurones. Note the general correspondence between the zones of binocular overlap and the domains of Col B, Col C, MLG and FLG neurones.

project ventrally to a median locus in ipsilateral protocerebrum.

The styles of sexual dimorphism in the blowfly and housefly show interesting differences. Neurones analogous to MLG 1, MLG 3 and Col C are found in the lobulae of male houseflies[5]. However, there is no single element corresponding to MLG 2: instead, the housefly has a small cluster of columnar cells (Col D[5], Fig. 2) collectively covering the same domain of the lobula. Like MLG 2 of *Calliphora*, their axons project ventrally into the ipsilateral rear protocerebrum. No neurones corresponding to FLG 1-4 were found in female houseflies although they were sought with the cobalt method, with reduced silver stains, and by reconstruction from serial 3-μm Araldite-embedded sections stained with methylene blue[5].

Comparisons between the sexes of *Calliphora* lead to the following conclusions. Three unique neurones in the male and female are probably analogous. The location of dendrites is similar and the cell body locations of two cells (MLG 1, 2 and FLG 1, 2) are similar. Similarly shaped neurones in males and females send axons to the same regions. Comparable neurones differ with respect to morphology, axon diameter, dendritic domain and layer relationships. These differences may reflect different synaptic connections in males and females. Other neurones (FLG 4 and Col C) are present in one sex but apparently have no analogue in the other sex.

The sexual dimorphism of lobula neurones can be summarised by projections of their dendritic domains out to the retinal surface, as in Fig. 3. This figure also compares regions of binocular overlap in males and females[9]. The overlap is greatest in the upper frontal retina of the male where there is also an extra zone of ommatidia not present in the female[10-12]. The MLG 1 and 2 fields, and the Col C assembly, correspond to this zone (Fig. 3). In the female, FLG 1 and FLG 2 cover slightly more of the retina than the female binocularity zone. In both sexes the strip of Col B neurones corresponds to the binocular overlap zone more ventrally. In females, FLG 3 and 4 lie within this zone whereas in males the ventral part of the MLG 3 domain is significantly wider than this zone.

It has been suggested that the upper front part of the eye of male houseflies may play a significant role in visually guided sexual behaviour[3]. Are the sexually dimorphic neurones important in sexually dimorphic behaviour? Their large size should permit exploration of this question by electrophysiological methods.

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Feeding behaviour of tsetse flies infected with salivarian trypanosomes

L. Jenni*, D. H. Molyneux†, J. L. Livesey‡ & R. Galun§

* Swiss Tropical Institute, Socinstrasse 57, CH-4051, Basle, Switzerland
† Department of Biology, Salford University
‡ Department of Aeronautical and Mechanical Engineering, Salford University, Salford, UK
§ Department of Zoology, Hebrew University, Jerusalem, Israel

Although much is known about factors which determine infection rates of salivarian trypanosomes (subgenera *Nannomonas*, *Duttonella* and *Trypanozoon*) in the tsetse fly *Glossina*[1,2], it is not clear why infection rates of *Trypanozoon* are high in mammalian hosts but low in wild-caught *Glossina*[3,4] and why trypanosomiasis occurs where *Glossina* is not readily detectable. We report here that the feeding behaviour of trypanosome-infected *Glossina* differed from that of uninfected control flies. Infected flies probed more frequently and fed more voraciously. We describe a specific relationship between trypanosomes and the mechanoreceptors responsible for detecting the rate of blood flow[5-7], and show how infection affects that rate in the labrum. We suggest that the observed differences in feeding behaviour result from impaired function of the labral mechanoreceptors in infected *Glossina*.

Glossina m. morsitans and *Glossina austeni* were obtained as pupae. Teneral *G.m. morsitans* were fed on mice (ICR) infected with *Trypanosoma* (*Trypanozoon*) *brucei* subsp. (ST1B 247) isolated in Serengeti, Tanzania, from *Alcelaphus buselaphus* (hartebeest) in 1971 and cryopreserved after one passage in the rat. Control flies received an uninfective blood meal at the same time and all flies were subsequently maintained as described previously[8]. Infected flies were detected by inducing individuals to probe on warmed slides 14 days after the infective feed and then feeding them on mice to verify their infectivity.

Forty infected and forty uninfected flies (20 males and 20 females in each group) were placed individually in glass tubes

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Table 1 Probing and feeding patterns of infected and uninfected *Glossina m. morsitans*

	Starving for 24 h			Starving for 48 h		
State of flies	No. feeding	No. feeding on first probe	Mean no. of probes ± s.e. before feeding	No. feeding	No. feeding on first probe	Mean no. of probes ± s.e. before feeding
40 Infected flies	40	3	5.08 ± 0.40	40	4	4.68 ± 0.37
40 Uninfected flies	28	13	1.89 ± 0.21	38	24	1.53 ± 0.13

The flies were 20–35 days old.

Table 2 Factors by which the required pressure drop $-\Delta\bar{P}$ would need to be increased for a given proportion of length colonised by trypanosomes $m(L)$ and for a given proportionate reduction in diameter $n(D)$ as a result of trypanosome infection if the same flow rate in the labrum was to be maintained

Proportion of diameter unoccluded	Proportion of length infected									
$n(D)$ \ $m(L)$	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
1.0	—	—	—	—	—	—	—	—	—	1
0.9	1.052	1.10	1.16	1.21	1.26	1.31	1.37	1.42	1.47	1.52
0.8	1.14	1.29	1.43	1.58	1.72	1.86	2.01	2.15	2.30	2.44
0.7	1.31	1.63	1.95	2.27	2.58	2.90	3.22	3.53	3.85	4.16
0.6	1.67	2.34	3.01	3.69	4.36	5.03	5.70	6.37	7.04	7.72
0.5	2.5	4	5.5	7	8.5	10	11.5	13	14.5	16
0.4	4.81	8.61	12.42	16.23	20.03	23.84	27.64	31.45	25.26	39.06
0.3	13.25	25.49	37.74	49.98	62.23	74.47	86.72	98.97	111.21	123.4
0.2	63.4	125.8	188.2	250.6	313	375.4	437.8	500.2	562.6	625
0.1	1,000.9	2,000.8	3,000.7	4,000.6	5,000.5	6,000.4	7,000.3	8,000.2	9,000.1	10,000

with netting at one end. Mice were anaesthetised with Nembutal before being used as the source of a blood meal. We first counted the number of probes by individual uninfected and infected flies (previously starved for 24 and 48 h) which pierced the skin with the haustellum before they were gorged. Table 1 shows that infected flies probed significantly more frequently than uninfected control flies, and suggests that infected flies were more voracious, for all 40 infected flies took blood after 24 h whereas only 28 of 40 uninfected flies did so.

We then offered 10 infected flies (5 male, 5 female) a series of mice on which to probe. If an individual fly failed to engorge after probing and withdrawing its haustellum, it was offered another mouse. Of a total of 41 probes by 10 infected flies, 38 proved to be infective to mice; the highest number of infective probes by an individual fly was 6. There was no significant difference between male and female fly behaviour in the above experiment.

The labrum of two *T.* (*T.*)*brucei*-infected *G.m. morsitans* were successfully prepared for scanning electron microscopy (SEM) out of a total of eight flies with mature infections examined with the light microscope. All eight flies had groups of trypanosomes in the proximal part of the labrum and SEM examination of two infected labra demonstrated a specific association between parasites and LCl mechanoreceptors (Fig. 1). We know the subgenera *Nannomonas* and *Duttonella* colonise the labrum where LCl mechanoreceptors are located[5] and are in close association with them[9,10].

We then applied principles of fluid mechanics[11–15] to predict the behaviour of blood in the labrum of *Glossina* to evaluate the likely effects of colonies of trypanosomes on the rate of blood flow. The data, units and sources which allow calculations of the predicted effects of parasites on flow in this system are available from the authors. The flow is characterised by the Reynolds number[12], and a frequency parameter[13–15] for the pulsating flow and non-newtonian viscosity effects are considered[11]. From these calculations, the pressure drop ($-\Delta\bar{P}$) below atmospheric required to obtain the same flow rate, $\bar{Q}$, for the meal is obtained. Table 2 indicates the factors by which $-\Delta\bar{P}$ would need to be increased for a proportion of the length mL (for m, 0.1–1.0) infected with trypanosomes and the proportionate unoccluded diameter $n\bar{D}$ (for n, 0.1–1.0) if the same meal flow rate were to be obtained. Alternatively, if $-\Delta\bar{P}$ remained constant, the flow rate would be inverse to the factors in Table 2. To illustrate the effect of occlusion due to *Nannomonas* and *Duttonella*[9,10], Table 2 shows that colonies of trypanosomes will be significantly invasive hydrodynamically, affecting blood flow rate through the labrum. Such changes would be detected by LCl mechanoreceptors[5]. Table 2 is also applicable to the trypanosome-infected hypopharynx, where a greater proportionate reduction in diameter would apply due to the smaller internal diameter of the hypopharynx; thus, the pressure required to expel saliva would be greater in infected than uninfected flies.

Table 3 The relationship between viscosity and feeding rate in *Glossina austeni* fed 24–48 h after emergence through bat or mouse skin membrane

Diet	Viscosity (cP)	Meal weight (mg)	Average feeding time (s)	Feeding rates (mg s^{-1})
10^{-3}M ATP saline	0.68	18	36	0.500
10^{-3}M ATP saline 3% dextran	1.36	18.6	48	0.387
10^{-3}M ATP saline 6% dextran	2.64	16.5	55.5	0.297
10^{-3}M ATP saline 12% dextran	6.78	18.1	82.25	0.220
Defibrinated sheep blood	3.10	16.5	63.3	0.26
Sheep plasma + 10^{-3}M ATP	1.15	16	37.1	0.431

Twenty flies were used for each diet and duration of feeding was measured electronically as described in ref. 6. Blood meal size was determined by weighing the fly before and immediately after feeding, before primary excretion took place. Viscosity was measured in a Cannon–Lebbelohde semi-microviscometer, size 200 at 38 °C

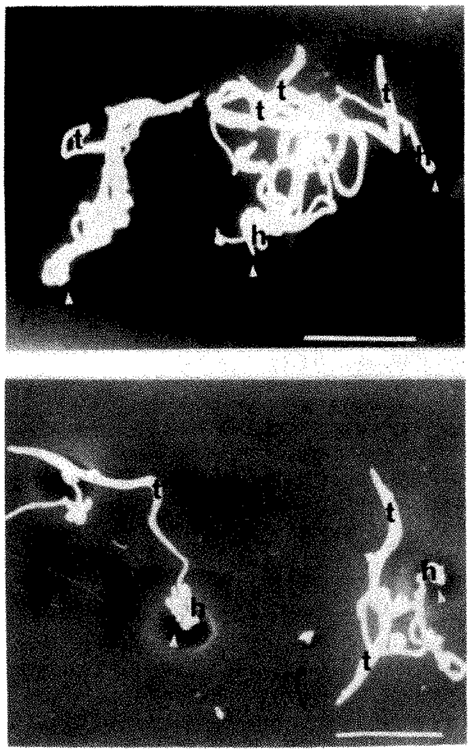

Fig. 1 Upper, scanning electron micrograph of *T. b. brucei* parasites (t, procyclic trypomastigotes) associated with two LCl mechanoreceptors (basal pits arrowed) in the proximal part of the labrum of *G. m. morsitans* 19 days after an infective feed. The preparation was fixed in 0.2 M glutaraldehyde in cacodylate buffer, critically point dried and examined in a Cambridge stereoscan. Scale bar, 10 μm. Lower, two procyclic parasites (t) associated with one LCl mechanoreceptor in the labrum of *G. m. morsitans* (and a single parasite associated with a second LCl). Scale bar, 10 μm.

However, if the viscosity of the blood meal was increased in membrane feeding experiments with *G. austeni*, there was a reduction in the rate of feeding of approximately 50% for a 10-fold increase in viscosity (Table 3). We have shown that there will be a need to increase either pump frequency or capacity by the factors given in Table 2 if the flow rate is to remain constant for any given occlusion in effective diameter by trypanosomes. However, we also know the pump performance is limited in its capacity to respond to an experimentally increased viscosity. Thus, to obtain the same blood meal size the infected fly would take longer to engorge or, if it did not take sufficient blood, would need to feed more frequently.

We know[5-7] that the feeding and gorging responses of *Glossina* are determined by chemo- and mechano-sensilla located in the labellum, labrum and cibarium. The LR7 sensilla of the labellum initially detect a suitable food source in their role as ATP detectors; probing continues until there is (1) sufficient stimulus of the LCl mechanoreceptive sensilla of the labrum and of the LC2s of the anterior cibarial wall by an adequate blood flow rate, at which time (2) the four LC4 sensilla of the posterior cibarial wall are stimulated by the ATP present in the meal to begin the gorging phase of the feeding cycle. Thus, impairment of the LCl mechanoreceptor function will affect sensory input and modify the feeding behaviour of infected flies as described above. Such behaviour indicates an advantageous adaptation by parasites that may have profound epidemiological and epizootiological implications. In particular, the significance of salivarian trypanosome infection rates in *Glossina* will require re-examination if studies on the behaviour of different tsetse species in the field confirm our laboratory findings.

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Larval hatching from vitellogenin-deficient eggs developed in male hosts of the silkworm

Okitsugu Yamashita & Ken Irie

Laboratory of Sericultural Science, Faculty of Agriculture,
Nagoya University, Chikusa, Nagoya 464, Japan

Egg production in insects is generally considered to require the availability of the yolk precursor, vitellogenin. In most insects, the synthesis of this protein in the female fat body and its uptake into the ovaries are under the control of the hormones, juvenile hormone and ecdysone[1], which is somewhat analogous to the oestrogenic control in oviporous vertebrates[2]. Indeed, vitellogenin constitutes ~90% of total egg proteins in the cockroach, *Leucophaea maderae*[3], and ~40% in the silkworm, *Bombyx mori* (unpublished data). However, in some insects[4-9], rudimentary ovaries transplanted into male hosts are known to develop to mature eggs with chorion but lacking vitellogenin. *Drosophila melanogaster* is exceptional in that the ovaries themselves can produce vitellogenin, which accounts for its accumulation in ovaries transplanted into males[8]. Vitellogenin is believed to represent a reserve for use during embryogenesis[10,11]. However, experiments on *D. melanogaster*[12] and *B. mori* (unpublished data) demonstrated that vitellin was not used rapidly during embryonic development and persisted at a considerable level in the newly hatched larvae. These facts suggest that vitellogenin may not be essential for ovarian development or for embryonic development. Therefore, the present experiments were carried out in order to establish whether the vitellogenin-deficient eggs that matured in male hosts, if activated by artificial parthenogenesis[13], can compute embryonic and further post-embryonic development. We have found that they can.

Silkworms of a bivoltine race (Shunrei and Shogetsu) were programmed to produce diapause eggs by incubating the parental embryonic stage at 25 °C under continuous illumination. On the third day of the fifth instar, ovaries were transplanted into males, the testes of which had just been extirpated. The operated larvae metamorphosed into pupae in 9 days and into adults after a further 14 days, as did non-operated control animals. Mature eggs with chorion were taken from the newly emerged adults. Table 1 compares various characteristics of these eggs with those of eggs matured in females. The eggs from males were less numerous and slightly light in weight. The contents of amino acids, RNA and lipids were almost the same but the glycogen content was clearly higher in the eggs from males, and the protein content was about 20% lower than that of controls.

Polyacrylamide disc gel electrophoresis of the proteins of eggs from males demonstrated eight bands comparable with those

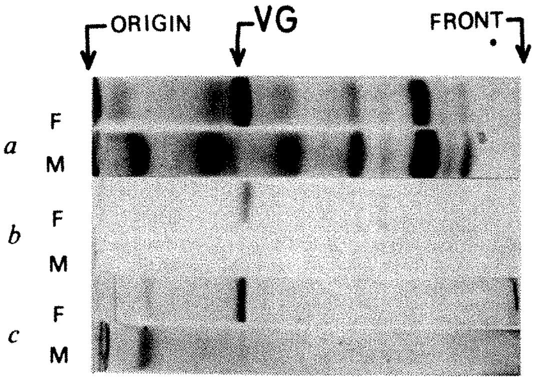

Fig. 1 Polyacrylamide gel electrophoresis of soluble proteins from eggs from females (F) and male hosts (M). Eggs (about 200 mg) obtained as described in Table 1 legend were homogenised in 5 vol of 0.4 MKCl in 50 mM phosphate buffer, *p*H 6.0. The homogenate was centrifuged at 15,000*g* for 15 min at 4 °C and 10–20 μl of the supernatant containing 0.002% bromphenol blue and 20% glycerol was applied to a 5% polyacrylamide gel (*p*H 8.5) and run at a constant current of 2 mA per gel[18]. Gels were stained[19] for protein with amido black 10B (*a*), for glycoprotein with Schiff's reagent (*b*), and for lipoprotein with Sudan black (*c*). VG, vitellogenin.

Table 1 Comparison of weight and composition of eggs developed in normal females and in male hosts

	Eggs developed in	
	Females	Male hosts
Wet weight (μg per egg)	600 ± 30	556 ± 11
Dry weight (μg per egg)	231 ± 1	188 ± 1
Amino acids (mg per g eggs)	2.3 ± 0.1	2.5 ± 0.2
RNA (mg per g eggs)	2.4 ± 0.8	3.0 ± 0.7
Lipids (mg per g eggs)	85.4 ± 2.4	87.8 ± 9.3
Glycogen (mg per g eggs)	26.5 ± 1.5	35.3 ± 2.5
Proteins (mg per g eggs)	105.2 ± 7.5	84.7 ± 10.7

Ovaries from fifth instar larvae were transplanted into males of the same stage. At the emergence of adults, ovaries were dissected out and mature eggs with chorion were collected by squeezing from ovarioles through a layer of gauze. After brief air drying, the eggs were weighed, then dried three times at 80 °C for 3 h to constant dry weights and weighed again. Fresh eggs were homogenised in cold water with an agate mortar and the homogenates were passed through a cotton pad to remove chorion residues. The filtered sample was used for determination of amino acids, RNA, lipids, glycogen and proteins. Amino acids were extracted three times with cold 5% $HClO_4$. After neutralisation with KOH, the solution was applied to a column of Dowex-50 (H^+ form) and eluted with 1 M NH_4OH. The pooled eluate was used for determination of total amino acids by the ninhydrin reaction, with leucine as standard[14]. From the acid-insoluble residues RNA was extracted with hot 5% $HClO_4$ and determined by the orcinol reaction, with yeast RNA as standard[15]. Protein was extracted from the residues with 0.5 M NaOH and determined by the method of Lowry *et al.*[16], with bovine serum albumin as standard. Lipids were extracted from fresh homogenates with methanol/chloroform (3:1) and the evaporated residues were then extracted with chloroform, washed with 0.5 M NaCl, dried and weighed[17]. For glycogen, the homogenate was digested with 30% KOH at 100 °C for 30 min, and glycogen was precipitated with 4 vol of ethanol overnight. In some experiments a cold $HClO_4$ soluble fraction was used directly for glycogen determination. Glycogen was determined with the phenol–sulphuric acid or concentrated H_2SO_4 method with glucose as standard[17]. Values are means ± s.e.m. from three to five determinations on different pools of eggs (about 350 eggs, 200 mg).

from normal eggs, but one normally major band corresponding to vitellogenin was lacking or at a trace level (Fig. 1). This was supported by staining for glycoprotein and lipid which are characteristic components of vitellogenin[1], and further by immunochemical assay (data not shown). Therefore, eggs from male hosts seem to differ from those produced in females chiefly in deficiency of vitellogenin.

To initiate embryogenesis in the eggs developed in male hosts, artificial parthenogenesis by a heat-shock treatment was carried out according to the method of Astaurov[13], who applied it to eggs from ovaries in females. The parthenogenetic development was easily detected in eggs by a reddish-brown pigmentation which reflected the formation of the serosal cells and entry into diapause (Fig. 2). The treatment induced parthenogenetic development in 63.1 ± 16.2% of the eggs from male hosts, and in 59.7 ± 0.8% of normal eggs from females. Thus, there was no appreciable difference in capacity for parthenogenetic response.

Because these eggs were programmed for diapause, diapause was then prevented artificially in the usual way by HCl treatment (Fig. 2). Embryonic development progressed and larvae of hatching size appeared in about 21.0 ± 4.2% of the coloured eggs 8 days after the HCl treatment. Hatching occurred in about 5.7 ± 2.7% of the parthenogenetic eggs, which is comparable with that in normal eggs from females[13]. The larvae appeared to be rather pale in colour but moved actively. When raised on fresh mulberry leaves, the larvae moulted to the second instar 4 days later, continued to grow succssfully and reached maturity to spin cocoons on day 25 at 25 °C.

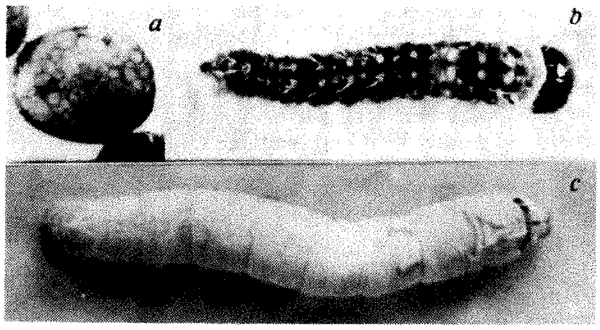

Fig. 2 Egg (*a*), newly hatched larva (*b*), and the fifth instar larva (*c*) from parthenogenetically activated eggs which had developed in male hosts. Ovaries dissected out from newly emerged males were immersed in water at 46 °C for 16 min and cooled abruptly in tap water for 10 min. After drying in air, the eggs were kept at 20 °C for 4 days to ensure parthenogenetic development[13]. The eggs with a reddish-brown colour were then soaked in HCl solution (specific gravity, 1.075 at 15 °C) at 47 °C for 3 min to prevent diapause[20]. They were kept at 25 °C and 75% relative humidity thereafter. After 12 days, larvae hatched and were raised on mulberry leaves. Egg and newly hatched larva, ×20; fifth instar larva, ×1.5.

We believe that the completion of embryogenesis and subsequent larval growth from the eggs that developed in male hosts is the first such case reported in insects as well as in other classes of animals. The results demonstrate that vitellogenin or vitellin is not essential for egg maturation and embryonic development. In addition, such a biological system should open a new field in reproductive and embryonic studies.

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Noradrenaline and cyclic AMP—independent growth stimulation in newt limb blastemata

Michel P. Rathbone & Jeannine Petri

Department of Neurosciences, McMaster University Medical Centre,
1200 Main Street West, Hamilton, Ontario, Canada L8S 4J9

Alfred F. Choo & David M. Logan

Department of Biology, York University, 4700 Keele Street, Downsview, Ontario, Canada, M3J 1P3

Robert L. Carlone & John E. Foret

Department of Zoology, University of New Hampshire, Durham, New Hampshire 03824

Adult newts regenerate functional limbs after amputation[1,2]. This process normally depends on the trophic influence of nerves on the regenerating limbs, particularly in the early stages before differentiation of the regeneration blastema[3], when it stimulates growth by maintaining high rates of macromolecular synthesis[4]. The sequence of biochemical events involved is unknown, but it has been suggested that intracellular cyclic AMP may be a second messenger within the blastema[5–10]. Many studies have indicated that the neural agent(s) involved might be protein[4,11–16]. The recent finding that blastemata contain high levels of catecholamines[17], however, has implicated noradrenaline (NA) as the neurotrophic agent, and suggested that it works via stimulation of β-adrenergic receptors on the blastemal cells, thereby raising the intracellular concentrations of cyclic AMP[10]. To test this hypothesis we studied the ability of NA alone and in combination with α-and β-adrenergic antagonists to increase cyclic AMP levels and to mimic the effects of nerves by maintaining high rates of protein synthesis[13,14] and high mitotic indices (MI)[15,16] in denervated blastemata in organ culture. We find that although NA raises cyclic AMP levels through a β-adrenergic effect, it does not maintain high rates of protein synthesis or high MI in cultured blastemata. It is unlikely therefore, that this hypothesis applies.

The rate of protein synthesis and the mitotic index in blastemata decrease after denervation[3,11]. Similar changes occur after blastemata are denervated and explanted into organ culture[13–16]. The addition of extracts of newt brains to the culture medium mimics the trophic effects of nerves by preventing these post-denervation decreases[13–16]. We cultured cone stage blastemata[18] from newts (*Notophthalmus viridescens*) in the presence of brain extracts, NA, and NA together with either an α- or β-adrenergic antagonists and determined the effects of these treatments on the cyclic AMP levels[10] rates of protein synthesis[13,14] and MI[15,16] in the blastemata. Histological examination revealed a normal, viable regenerate[16]. In confirmation of our previous findings, blastemata cultured in the presence of chick embryo or adult newt brain extracts had rates of protein synthesis and mitotic indices that were significantly greater than those in control blastemata cultured in their absence. In contrast to the effects of brain extracts on the blastemata, NA (at concentrations ranging from 10^{-6} to 10^{-4} M) did not elevate the MI and caused a small but significant decrease in the rates of protein synthesis below control values (Fig. 1). This indicates that, under the present experimental conditions, contrary to the hypothesis proposed by Taban *et al.*[10], it is unlikely that NA is an important neurotrophic agent since it does not mimic at least two important neurotrophic effects: maintenance of high rates of protein synthesis and high MI in the blastemata.

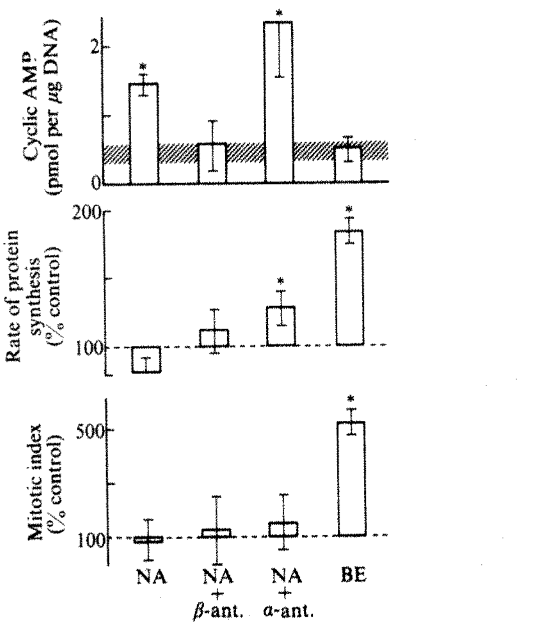

Fig. 1 Effects of NA, adrenergic antagonists and brain extracts on the cyclic AMP concentration, mitotic index, and protein synthesis of blastemata cultured *in vitro*. Cone stage blastemata were explanted and cultured as described previously[14–16]. Control cultures contained no addition to the medium; there were four treatment groups; NA = medium containing 10^{-4} M NA; NA + α-ANT = medium containing 10^{-4} M NA + either 10^{-4} M phenoxybenzamine or 10^{-4} M phentolamine; NA + β ANT = medium containing 10^{-4} M NA + 10^{-4} M propranolol; BE = medium containing an aqueous extract of adult newt brain (0.3 mg ml^{-1}) or embryonic chick brain (1.0 mg ml^{-1}) (refs 14–16). Blastemata were cultured in the presence of the various agents and the levels of cyclic AMP (at 10 min), rates of protein synthesis (at 12 h) and MI (at 24 h) were determined. At these time periods, the maximal effects of the treatments on various parameters occurred. Each value is the mean ± s.e.m. of six determinations. Cyclic AMP was determined using a radioimmunoassay[22] as modified by Harper and Brooker[23]. The rate of protein synthesis in the blastemata[14] and mitotic indices[15,16] were determined as described elsewhere. Differences in cyclic AMP concentrations, mean MI and rate of protein synthesis between experimental blastemata and controls from contralateral limbs were analysed for significance by the *t*-test for non-independent samples[24]. $^*P \leq 0.05$. The cross-hatched area on the cyclic AMP graph represents control values ± 1 s.e.m. The control values were as follows: MI = 0.64 ± 0.06% (s.e.m.). Rate of protein synthesis = 2.33 ± 0.05 (s.e.m.) pico atoms of carbon per g protein per 12 h. Cyclic AMP = 0.478 ± 0.06 (s.e.m.) pmol per μg DNA.

Unlike NA alone, NA in combination with the β-adrenergic antagonist propranolol had no effect on the rate of protein synthesis in the blastemata, or on their MI, both of which were the same as in control cultures (Fig. 1). Conversely, the addition to the cultures of NA plus either of the α-adrenergic antagonists, phenoxybenzamine or phentolamine, significantly increased the rate of protein synthesis in treated blastemata, while MI was unaffected. The increase in protein synthesis due to this β-adrenergic effect was far smaller however, than that produced by brain extracts (Fig. 1). This small increase in protein synthesis observed when the α-adrenergic effects of NA are inhibited, leaving only the β-adrenergic effects, is of particular interest. It is unlikely that the selective stimulation of β-adrenergic receptors is responsible for the neurotrophic effects on blastemata *in vivo* however, since there is no evidence that naturally occuring α-adrenergic antagonists exist. Furthermore, when the β-adrenergic effects of NA are blocked, protein synthesis in blastemata is not inhibited. It appears, therefore, that the inhibitory effects of NA on the rate of protein synthesis may be due to the combined simultaneous activation of α- and β-adrenergic receptors.

Although NA stimulated increases in cyclic AMP in cultured blastemata (and thus confirmed the report of Taban *et al.*[10]), it failed to stimulate increases in the rates of protein synthesis or

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mitosis, two neurotrophically regulated processes of the nerve-dependent growth phase of limb regeneration[3,11]. Previous studies have shown that the effects of brain extracts on protein synthesis and mitosis in cultured blastemata are similar to the effects of nerves on these processes *in vivo*[14,16]. Thus, the failure of NA to mimic the neurotrophic effects of brain extracts *in vitro* on these two processes would argue against this catecholamine as an important trophic effector during the early stages of regeneration. The possibility remains, however, that NA may play a significant role during the later nerve-independent stages of redifferentiation. Indeed, pharmacological studies on regeneration *in vivo* support such an hypothesis[7]; treatment with anti-adrenergic agents does not prevent blastema formation (a nerve-dependent process) but retards or represses subsequent morphogenesis (a nerve-independent process).

Additionally, crude brain extracts were ineffective in stimulating increased cyclic AMP levels in cultured blastemata. These results demonstrate that the elevation of intracellular cyclic AMP in blastemal cells is not invariably associated with increases in the rates of protein synthesis and mitosis in the blastemata. Consequently, the previously described stimulation of DNA and protein synthesis and mitosis by exogenous dibutyryl cyclic AMP[5,16,19] may be mediated through a different mechanism.

We have previously found that stimulation of α-adrenergic receptors increases cyclic GMP levels in newt tissues, and that β-adrenergic stimulation inhibits such increases[20]. It has been suggested that the ratio of cyclic AMP to cyclic GMP may play an important role in the response of tissue muscle to neurotrophic influences[10,21]. If true, this might explain the observed failure of NA to elevate protein synthesis and MI. This interpretation is not supported by observations of the effects of denervation on the levels of cyclic nucleotides in blastemata *in vivo* (M.P.R. Liversage and McLaughlin, unpublished observations); it appears likely that any alterations in cyclic AMP and cyclic GMP levels after denervation are not induced directly by the neurotrophic stimulus, but rather reflect a more general metabolic alteration in the blastemata that is part of the overall metabolic response associated with the increased growth stimulated by the neurotrophic influence.

Thus our findings are inconsistent with the hypothesis[10] that nerves transmit trophic information to blastemal cells via β-adrenergic receptor mediated stimulation of adenylate cyclase with subsequent production of cyclic AMP. Studies are currently in progress to determine the nature of the neurotrophic influence and to elucidate the molecular mechanisms involved in the response of blastemata to neurotrophic stimuli.

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Cigarette smoke-induced DNA damage and lung cancer risks

J. M. Hopkin* & H. J. Evans

Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, UK

Epidemiological studies have firmly established that cigarette smoking causes almost all cases of anaplastic and squamous cell bronchial carcinomas[1]. It is equally well known, however, that many smokers do not develop lung cancer and, although the amount of tobacco smoked is undoubtedly the dominant risk factor, it seems possible that other factors may also be involved[2]. There is increasing evidence to support Boveri's[3] old hypothesis that somatic mutation is an important event in the development of cancer[4,5] and we have recently shown that cigarette smoke condensate (CSC) produces many dose-related lesions in the cellular DNA of cultured human lymphocytes as measured by sister chromatid exchange (SCE) induction[6]. Cytogenetic studies[7,8] had previously shown an increase in chromosomal aberrations in blood lymphocytes of heavy smokers relative to non-smokers, but little[9] or no[8,10] increase in SCEs. We have now measured basal and CSC-induced SCE rates in lymphocytes from different individuals and report that smokers have higher rates than non-smokers and that smokers with untreated lung cancer have consistently higher rates than their matched heavy smoking controls. These results are in keeping with the somatic mutational hypothesis and the epidemiological evidence, but also raise questions related to difficulties in smoking dosimetry and to possible variation among different individuals' responses to a common insult.

The responses of cultured lymphocytes from four groups of people to four doses of smoke condensate were examined by the visual scoring of induced SCEs. We used the same methods as in our original report[6] and, as before, samples were coded and scored 'blind'. Smoke condensate produced from a standard brand of British cigarette was dissolved in dimethylsulphoxide at serial dilutions and stored in small aliquots at −20 °C. This condensate, together with single stocks of culture medium and fetal calf serum, were used throughout the series. Four cultures, each of 10 ml and containing 0.8 ml of whole blood with an average of 10^6 lymphocytes, were set up from each individual. The four groups studied were: *A*, 10 healthy smokers, none of whom to our knowledge had been unduly exposed to any mutagen other than cigarette smoke. *B*, 10 healthy non-smokers who were examined in pairs matched for age and sex with members of group *A*. *C*, 12 patients with histologically proven but untreated non-disseminated anaplastic or squamous cell bronchial carcinomas, in whom diagnostic biopsies had been obtained by fibreoptic bronchoscopy under local anaesthetic. Ten of these patients were continuing to smoke at the time of testing (C_1), but two had stopped smoking 2 and 5 yr previously (C_2). *D*, 10 control patients with other disorders, but none with any form of cancer, who were on average 10 yr older and had smoked similar numbers of cigarettes per day, but for 10 yr longer, than our lung cancer patients. All individuals in groups *C* and *D* were inhaling smokers and smoked their cigarettes to comparable butt lengths. Only one individual had been exposed to a recognised occupational risk factor (asbestos). All were hospital inpatients, none of whom was acutely ill, and individuals from both groups had received similar exposure to X rays and drugs.

From our results, shown in Tables 1 and 2, it may be seen that cigarette smoke condensate (CSC) present in small quantities

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* Present address: Department of Medicine, University of Birmingham, Queen Elizabeth Hospital, Birmingham, UK.

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Table 1 Frequency* of sister chromatid exchanges in cultured lymphocytes from healthy smokers and non-smokers exposed to cigarette condensate (CSC)

	Age	Sex	Cigarettes per day	Dose of CSC†			
				0	0.1	0.5	1.0
B Non-smokers	47	M		7.5	9.4	11.6	13.6
	45	F		7.2	8.4	11.1	16.8
	29	F		7.1	8.0	12.7	15.8
	24	M		7.8	9.5	11.6	16.0
	21	M		7.5	7.6	12.7	15.8
	75	M	(ex-smoker of 17 yr)	7.0	9.9	12.2	18.3
	73	F		7.0	9.1	12.7	19.4
	59	M		7.2	8.2	15.1	18.0
	48	M		8.5	9.5	12.8	15.2
	54	F		7.5	9.1	14.0	17.9
	47.6			7.43 (0.14)	8.87 (0.24)	12.65 (0.38)	16.68 (0.55)‡
A Smokers	47	M	15	6.6	9.7	13.4	15.4
	54	F	20	7.3	11.7	14.1	17.7
	32	F	20	8.5	11.9	15.3	19.0
	24	M	15	8.2	10.5	14.0	16.5
	20	M	15	6.8	10.0	12.8	15.8
	70	M	10	8.0	13.8	17.0	22.3
	75	F	12	10.5	11.9	16.2	—
	64	M	10	6.5	7.5	11.1	16.9
	53	M	35	9.4	12.0	15.1	17.5
	54	F	30	12.0	14.6	17.5	20.1
	49.3		18.3	8.38 (0.57)	11.36 (0.65)	14.62 (0.64)	17.91 (0.74)§
				9.30 (0.89)	12.55 (0.65)	15.5 (0.72)	18.53 (0.60)‖
				7.76 (0.62)	10.57 (0.79)	14.08 (0.89)	17.38 (1.26)¶

* Mean of 20 cells per culture.

† CSC, mg per 10 ml culture containing 10^6 lymphocytes (diluent, dimethyl sulphoxide 20 μl). Note: 0.1 mg CSC ≡ 1/400 of smoke from one high tar cigarette; 0.5 mg CSC ≡ 1/80; 1.0 mg CSC ≡ 1/40.

‡ Mean (s.e.) of individual means.

§ Mean (s.e.) overall.

‖ Mean (s.e.) ≥ 20 cigarettes per day.

¶ Mean (s.e.) < 20 cigarettes per day.

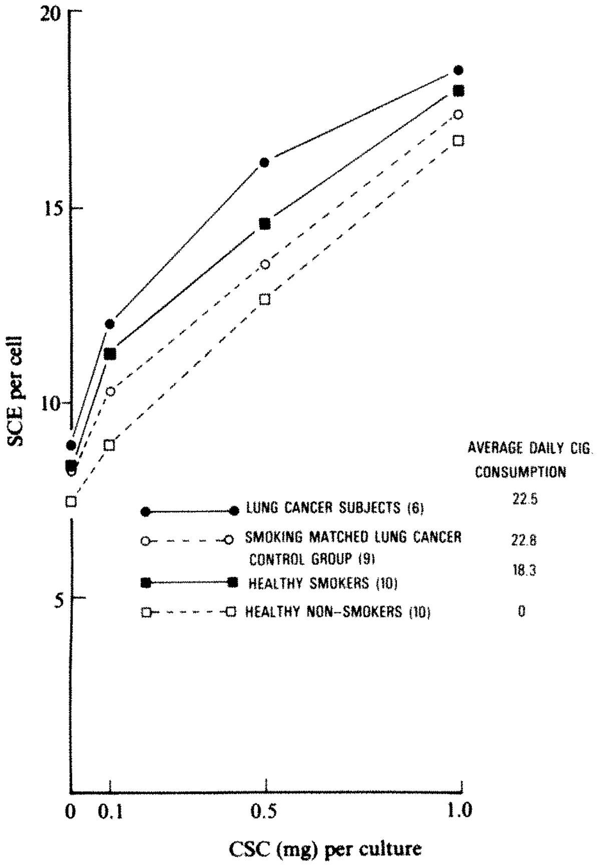

Fig. 1 Within group pooled mean SCEs (that is, the mean of individuals' means), at different dose levels of CSC, for the various groups.

produced dose-related increases in SCEs in lymphocytes from all individuals. Basal and induced SCE rates did, however, differ significantly among individuals.

Healthy smokers (*A*) had, relative to healthy non-smokers (*B*), higher mean basal and also higher induced SCE rates (following exposure to 0.1 mg of CSC, $P<0.05$) and the heaviest smokers had the highest SCE rates. A comparative study on cultured whole blood cells of two smokers and two non-smokers showed that these differences between smokers and non-smokers are maintained even in the absence of autologous plasma; we conclude, therefore, that the greater number of SCEs produced by condensate in smokers' cells in comparison with non-smokers must reflect some cellular alteration dependent on previous exposure to smoke *in vivo*.

For patients with lung cancer who were continuing to smoke (C_1), SCE rates were significantly higher at all levels of testing than for the smoking matched cancer controls (*D*). During final analysis, we found that there were more plain cigarette (middle or high tar) smokers at the time of testing in our lung cancer group than in the matched controls and this might have introduced a bias. However, exclusion of these plain cigarette smokers from both groups does not influence the result and smoking lung cancer subjects continue to have higher SCE rates at all levels of testing (0.1 or 0.5 mg of CSC, $P<0.01$). The two ex-smokers with lung cancer (C_2) had responses indistinguishable from those of healthy non-smokers, suggesting that high SCE rates are not secondary to the development of cancer. Furthermore, the overlapping of responses between the healthy smokers (*A*), the smoking matched non-cancer patients (*D*) and the smoking lung cancer patients (C_1), supports this conclusion.

Figure 1 presents mean basal and induced SCE frequencies for filter cigarette smokers in groups *A*, *C* and *D* (that is, smokers exposed to comparable levels of smoke *in vivo*) and for

Table 2 Frequency of sister chromatid exchanges in cultured lymphocytes from patients with lung cancer and matched smoking controls exposed to • CSC

	Age	Sex	Cigarettes per day	Cigarette type & smoking duration		Dose of CSC			
				Plain	Filter	0	0.1	0.5	1.0
C_1 Lung cancer subjects	60	M	40	47	—	12.4	14.1	16.5	21.6
	51	M	25	36	—	9.6	12.2	16.8	20.4
	66	M	20	58	—	12.4	15.8	21.0	24.6
	51	M	25	36	—	12.3	14.0	20.3	20.1
	63	M	30	36	20	8.5	12.4	15.5	19.8
	55	F	20	23	15	7.3	13.2	14.3	16.3
	63	F	20	25	15	9.9	10.8	15.9	20.4
	65	M	25	39	12	8.3	13.8	19.0	20.3
	53	F	20	18	20	9.8	11.4	16.5	17.8
	45	M	20	—	25	9.8	11.3	15.6	16.5
	56.9		24.5	42.3 yr		10.03 (0.57)	12.9 (0.49)	17.14 (0.70)	19.73 (0.78)*
						11.7 (0.69)	14.03 (0.74)	18.65 (1.17)	21.67 (1.02)†
						8.93 (0.44)	12.10 (0.40)	16.13 (0.64)	18.43 (0.75)‡
C_2 Lung cancer subjects (ex-smokers of 2 and 5 yr)	57	M	30	20	20	7.3	9.5	10.4	12.7
	56	M	20	20	11	7.5	9.1	12.0	14.4
D Smoking matched cancer control group	56	M	30	42	—	10.6	13.9	18.0	19.2
	65	M	20	40	12	8.7	10.8	13.8	—
	85	M	20	55	20	8.6	10.1	12.9	—
	73	M	30	43	20	7.2	10.8	13.9	17.0
	61	F	20	28	15	9.4	11.2	13.2	16.7
	64	F	20	34	15	8.6	10.6	15.9	21.9
	73	M	25	44	15	7.7	8.3	13.1	18.4
	58	F	20	33	10	8.6	11.4	13.1	15.0
	71	M	30	56	2	6.9	8.6	12.2	15.7
	53	M	20	17	20	8.8	11.1	13.8	16.8
	65.9		23.5	52.1 yr		8.51 (0.33)	10.68 (0.49)	13.99 (0.54)	17.59 (0.77)§
						8.28 (0.27)	10.30 (0.38)	13.54 (0.34)	17.35 (0.85)‖

* Mean (s.e.) overall.
† Plain cigarette smokers at testing.
‡ Filter cigarette smokers at testing.
§ Mean (s.e.) overall.
‖ Filter cigarette smokers at testing.

non-smokers (*B*) and shows specifically that there is a consistent ordering of our four groups at all levels of testing. Superficially, the almost parallel dose–response curves in Fig. 1 might imply that the increased SCE rates following *in vitro* induction may simply emphasise *in vivo* differences, with similar increments being added for all four groups for each *in vitro* unit dose. This cannot be ruled out, but at lower CSC doses the relative response is not uniform between groups and more data would be needed to distinguish between these two possibilities. More important, however, is the fact that *in vitro* induction highlights the differences between the four groups. Highest responses were found for smokers with lung cancer (C_1). Among smokers, the lowest responses were found for the older heavy-smoking non-cancer patient group (*D*), and this is in keeping with our selection of these individuals as a 'relatively low risk' group. An unselected group of healthy and younger smokers (*A*) who might reasonably be expected to contain individuals of varying risk for lung cancer show a mean response intermediate between the lung cancer subjects (C_1) and their controls (*D*). This association of risk with extent of DNA damage supports the possibility that somatic mutation may be important in initiating malignant transformation in cells.

Although we have shown that cigarette smoking and the number and type of cigarette smoked significantly influence SCE rates, it is also clear that smokers seemingly exposed to similar amounts of cigarette smoke *in vivo* can have significantly different SCE rates (groups C_1 and *D*). These differences might simply result from discrepancies in actual amounts of tobacco smoked—certainly, all smoking histories describe only alleged amounts—or from varying patterns of inhalation or of airflow in the bronchial tree, both of which would clearly influence the dose of carcinogen delivered to bronchial mucosa and peripheral lymphocytes. Alternatively, if real smoke dosage *in vivo* was equivalent in both groups—and there is no way of determining this precisely—these differences might reflect the influence of other factors on the extent of measured DNA damage. In the context of Tokuhata and Lilienfield's[11] finding of significant familial aggregation of lung cancer not extending to spouses, it seems possible that some of these factors might be genetic and this would not be surprising for complex events resulting in DNA exchange, where presumably many enzymatic processes are involved, particularly in a species like man, where high rates of protein polymorphism are known to exist[12].

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Peptide-containing neurones connect the two ganglionated plexuses of the enteric nervous system

**Kristjan R. Jessen, Julia M. Polak*,
Susan Van Noorden*, Stephen R. Bloom†
& Geoffrey Burnstock**

Department of Anatomy and Embryology, University College London, Gower Street, London WC1

* Department of Histochemistry,
† Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12, UK

The enteric nervous system (ENS) of the mammalian gut consists principally of two ganglionated plexuses, the myenteric and submucous, which are embedded in the gut wall[1]. Little is known about the anatomical and functional connections between the two plexuses and further clarification of their relationship is of central importance for the understanding of the ENS. In the present study we have approached this problem in a new way, using methods we have previously developed for maintaining the myenteric and submucous plexuses separately *in vitro* for several weeks[2,3]. In an immunohistochemical study of these preparations and also of sections from normal and extrinsically denervated gut wall, we have found evidence for reciprocal projections between the myenteric and submucous plexuses, by nerve fibres containing two putative neurotransmitters, vasoactive intestinal polypeptide (VIP)[4] and substance P (ref. 5) (see Fig. 1). Our observations were supported by radioimmunoassay of tissue extracts. These results suggest that one of the roles of these peptides in the gut is to coordinate the function of the two enteric plexuses.

The normal distribution of VIP and substance P immunoreactivity in sections through the wall of the guinea pig caecum underneath and including the taenia coli is illustrated in Fig. 2. The VIP antiserum revealed a dense network of mostly varicose nerve fibres surrounding the ganglion cells of the myenteric plexus. Fibres were also frequent in the longitudinal and circular muscle, running parallel to the long axis of the muscle bundles, as well as in the ganglia of the submucous plexus. In the submucosa outside these ganglia and in the mucosa, VIP immunofluorescent fibres were present, but infrequent. Occasionally immunofluorescent nerve cell bodies were seen in the ganglia of the submucous plexus. The distribution and density of substance P immunoreactivity was similar to that of VIP, except that the density of substance P immunofluorescent fibres in the ganglia of the myenteric plexus was somewhat higher than that of VIP fibres. Substance P immunofluorescent fibres were not seen in the mucosa and no nerve cell bodies were detected with certainty.

It is recognised that neuronal perikarya of peptide-containing neurones are difficult to visualise, even if the axons to which they give rise contain demonstrable peptide stores[6,7]. To identify unambiguously the intrinsic source of the peptide-containing fibres we used our ability to immunostain tissue culture preparations of the myenteric and submucous plexus. The plexuses were removed from the gut wall and maintained separately *in vitro* for 1–2 weeks as described previously[2,3]. This enabled us to study peptide immunoreactivity in varicose axons which grow out from the neurones of each plexus during the culture period; these plexuses are free of axons extrinsic to the gut and also of those intrinsic axons, which *in situ* might run between them. Immunostaining of the cultures revealed that no VIP immunofluorescence could be detected in the myenteric plexus, whereas explants of submucous plexus contained numerous intensely fluorescent varicose fibres, which could often be traced for long distances in the outgrowth zone around the explants, as well as being observed among the neuronal cell bodies inside the explant (Fig. 3*a*). Conversely, substance P immunofluorescence was absent from the explants of the submucous plexus but was found in abundance in varicose fibres of the myenteric plexus cultures, both in the outgrowth zones and inside the explants (Fig. 3*b*). It is unlikely that our inability to observe VIP immunoreactivity in the myenteric plexus cultures is due to the failure of VIP-containing neurones to grow *in vitro*, or to technical difficulties in demonstrating VIP stores in nerve fibres in culture, because of the large numbers of VIP fluorescent fibres found in the cultures of the submucous plexus. The same argument applies to the absence of substance P immunoreactivity from the submucous plexus cultures. We therefore suggest that the guinea pig caecum contains intrinsic systems of both VIP-and substance P-containing neurones, the intrinsic VIP fibres originating exclusively from cell bodies located in the submucous plexus and the intrinsic substance P fibres emanating only from cell bodies in the myenteric plexus.

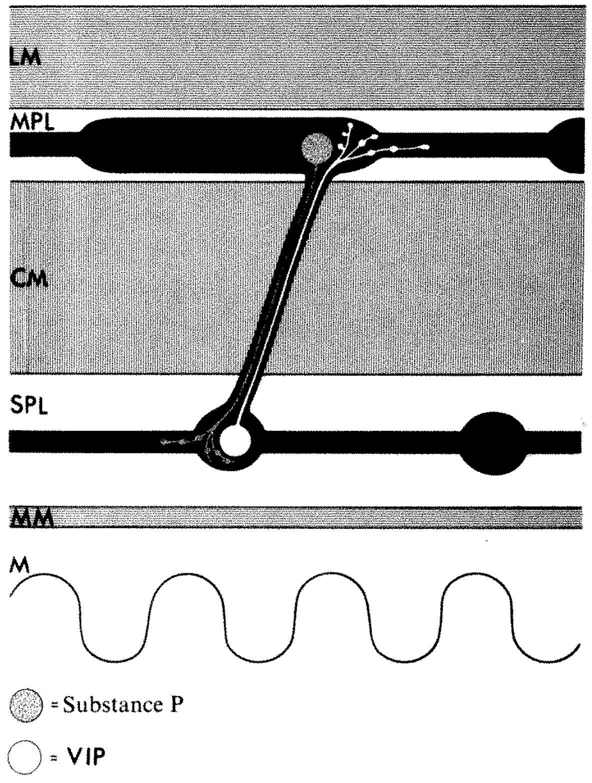

Fig. 1 Schematic drawing of a section through the wall of the caecum including the taenia, showing VIP- and substance P-containing neurones connecting the myenteric and submucous plexuses. LM, longitudinal muscle—this muscle layer constitutes the taenia coli. MPL, myenteric plexus—one whole ganglion and part of another are illustrated together with interconnecting strands. CM, circular muscle—the longitudinal and circular muscle layers are together referred to as the external muscle layer. SPL, submucous plexus—two ganglia and interconnecting strands are shown. MM, mucosal muscle. M, mucosa.

In the intact gut, however, we found VIP and substance P fibres densely surrounding cells in the myenteric and submucous plexuses respectively. There are two alternative explanations for these observations: (1) these fibres are extrinsic, originating from cell bodies outside the gut; or (2) the VIP fibres in the

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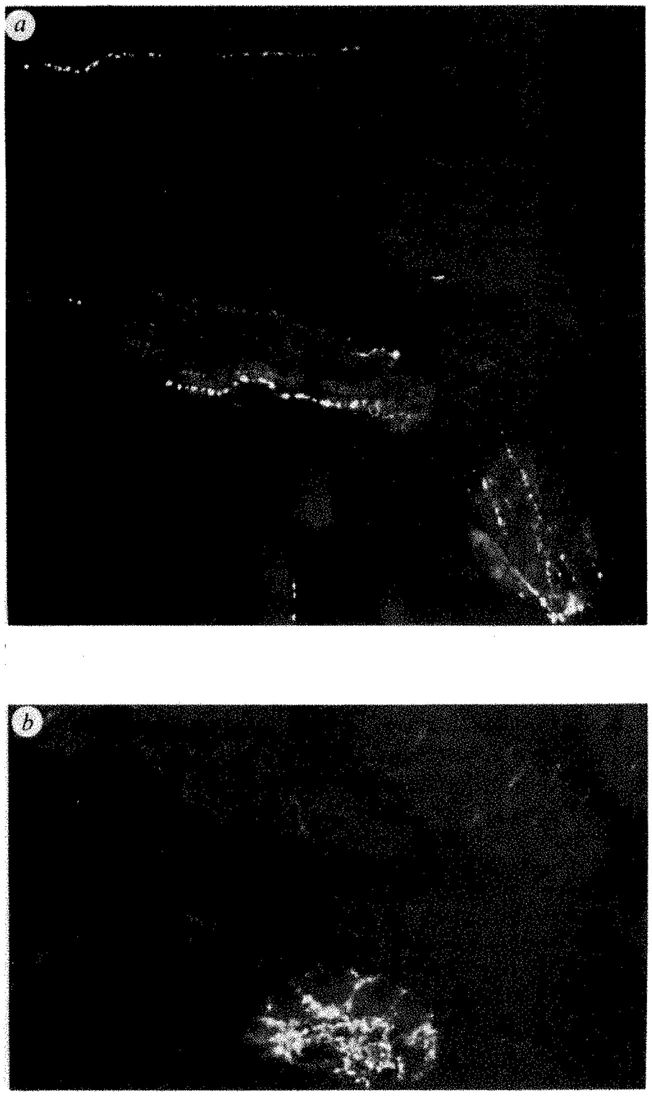

Fig. 2 Immunostaining of nerve fibres in sections from the caecum. *a*, VIP antiserum; *b*, substance P antiserum. In (*a*) immunoreactive beaded fibres can be seen among non-immunoreactive cell bodies in two ganglia of the myenteric plexus. A long beaded fibre is also visible in the longitudinal muscle above the ganglia and a short segment of a stained fibre can be seen associated with transversely cut bundles of the circular muscle below the ganglia (× 175). In (*b*) immunoreactive fibres can be seen surrounding non-reactive neuronal cell bodies in a ganglion of the submucous plexus. Above the plexus faint immunoreactivity can be seen in transversely cut fibres in the muscle bundles of the circular muscle layer (×250). Both VIP and substance P antibodies were raised in rabbits using purified porcine VIP or synthetic substance P coupled to bovine serum albumin by the carbodiimide method. The VIP antibody was directed to the C-terminal of the molecule and was used at a dilution of 1/400. The substance P antibody was directed to the whole molecule and was used at a dilution of 1/2,000. Neither antibody showed any significant cross-reactivity with other known neuropeptides. Staining was carried out by the indirect immunofluorescence method[14]. Tissue cultures or 10-μm thick cryostat sections of benzoquinone-fixed[15] specimens from the caecum were incubated with the peptide antibodies overnight at 4 °C. A second layer of fluorescent isothiocyanate conjugated anti-rabbit globulin was applied for 1 h at room temperature. In control experiments sections or cultures were incubated with nonimmune serum or VIP and substance P antisera which had been incubated with purified VIP or substance P respectively; no immunostaining was observed in these experiments.

myenteric plexus originate from the submucous plexus and the substance P fibres in the submucous plexus originate in the myenteric plexus. To distinguish between these possibilities we denervated the caecum of extrinsic nerve fibres by crushing the mesenteric nerves with fine forceps 3 weeks before killing, or, in some cases, by freezing them with a freeze probe. The effectiveness of the denervation was ascertained by noting the disappearance of the extrinsic adrenergic fibres from the gut wall (see ref. 8). No change in the amount or distribution of VIP or substance P immunofluorescent fibres was seen in the ganglia of the enteric plexuses in four experiments in spite of complete disappearance of adrenergic fibres. Furthermore, no significant change in VIP content, as measured by radioimmunoassay[9], was observed when denervated segments of gut wall underneath and including the taenia were compared to normally innervated segments from the same animal or from other control animals; the values for normal segments were 14.8 ± 4.2 pmol per g wet weight of tissue ($n = 6$) and for denervated segments 12.7 ± 4.3 pmol per g wet weight of tissue ($n = 4$). It is conceivable that nerve fibres may reach the caecum by running inside the gut

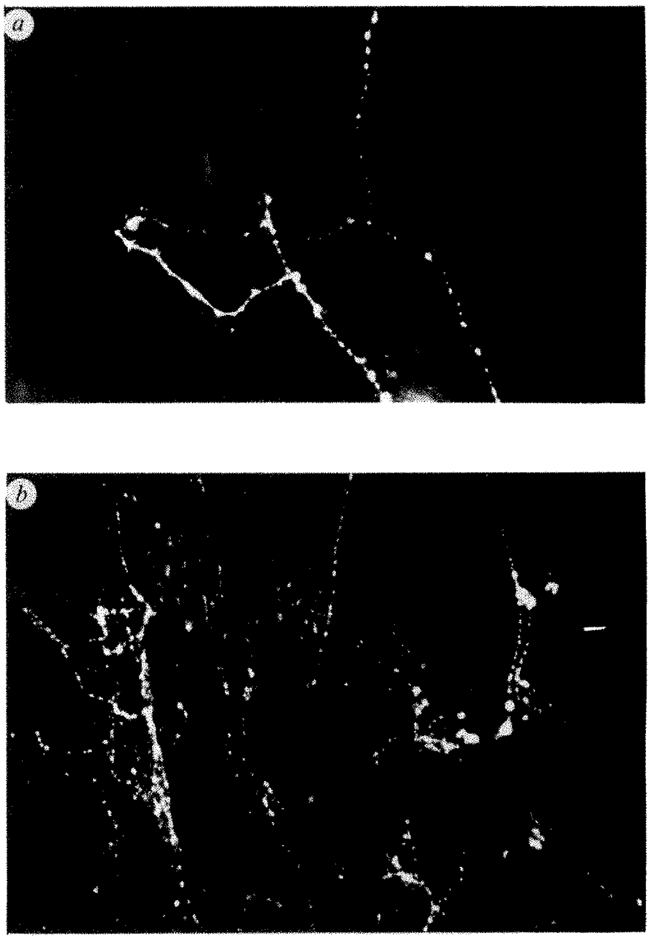

Fig. 3 Immunostaining of nerve fibres in tissue culture preparations of the enteric plexuses, *a*, VIP antiserum applied to a culture of the submucous plexus; intensely stained, apparently single, beaded nerve fibres, lying on top of non-immunoreactive, flattened, non-neuronal cells in the outgrowth zone of a culture which had been maintained for 7 days *in vitro* (×700). *b*, Substance P antiserum applied to a culture of the submucous plexus; several immunostained nerve fibres lie either apparently singly or in bundles in the outgrowth zone of a culture which has been maintained *in vitro* for 11 days. Under the nerve fibres lie flattened non-neuronal cells which show no immunoreactivity (×500).

wall, originating either from extrinsic nerves which enter the gut above or below the caecum or from the enteric plexuses of the small intestine or proximal colon, although the existence of such fibres has never been demonstrated. These fibres would not be affected by our denervation procedures. We therefore made a circumferential cut through the external muscle of the guinea pig caecum in the region just below the site of entry of the small intestine and proximal colon. The incision extended to the submucosa without penetrating it. In this way all nerve fibres which might reach the myenteric plexus of the caecum via the muscle layers or via the myenteric plexus were severed. The animal was killed 18 days later and sections from the caecum were immunostained, revealing VIP and substance P immunostaining which was identical both in distribution and density to that of normal material. Taken together these experiments demonstrate that the caecum receives no significant extrinsic innervation by VIP- or substance P-containing fibres.

We thus conclude that the submucous plexus of the caecum contains VIP neurones which provide most or all of the VIP innervation found in all layers of the intestinal wall in this area of the gut, including the dense network of fibres in ganglia of the myenteric plexus. In contrast, the substance P immunoreactive fibres of the gut wall, which are present in highest density in the ganglia of the myenteric and submucous plexuses, originate mostly or exclusively from the neurones located in the myenteric plexus. We have shown that in the caecum no VIP fibres originate from the myenteric plexus, and no substance P fibres emanate from the submucous plexus. This provides evidence for a VIP-containing pathway running from the submucous plexus to innervate neurones in the myenteric plexus as well as for a substance P-containing pathway providing innervation by the myenteric plexus of the submucous plexus as shown schematically in Fig. 1. A suggestion that there might be nerve connections between the two plexuses has been made previously on the basis of light microscopial findings[10], and has since received some support from physiological experiments[11]. Note, for comparison, that enkephalin immunofluorescent fibres do not span the two ganglionated plexuses but originate in the myenteric plexus and are confined to its ganglia and the external muscle layer[12,13]. A detailed account of the distribution of VIP, substance P and enkephalin neurones in the guinea pig small and large intestine is in preparation[13].

Although variations of the pattern described here may exist in other parts of the gut (for instance, some VIP-containing fibres originate from the myenteric plexus of the ascending colon and small intestine[13]) and in other species, these observations warrant pharmacological and physiological investigations into the possibility that one of the roles of VIP- and substance P-containing neurones in the gut is to provide functional connections between the two enteric plexuses.

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Substance P increases membrane conductance in parotid acinar cells

D. V. Gallacher* & O. H. Petersen†‡

* Glasgow Dental Hospital and School, 211 Renfrew Street, Glasgow, UK
† Department of Physiology, The University, Dundee, UK

Substance P, a naturally occurring polypeptide of mammalian origin[1], has been implicated both as a neurotransmitter and a neurohormone[2]. However, little is known of the ionic mechanisms underlying the postsynaptic response to substance P. In myenteric neurones Katayama and North[3] reported substance P-evoked depolarisations (of varying latency) associated, surprisingly, with a decrease in membrane conductance. A direct measurement of reversal potential in normal ionic conditions was not achieved but it was suggested that substance P acts by reducing membrane potassium conductance. In contrast, work on salivary glands suggests that substance P evokes an increase in potassium conductance[4,5]; however, electrophysiological work has not been carried out to verify this. We report here that substance P evokes a marked increase in rat parotid acinar cell membrane conductance associated with a potential change (latency 1.7 s) that reverses at about −65 mV. The reversal potential for substance P is shown to be identical to that obtained in the same cells for acetylcholine (ACh) and adrenaline. The identical membrane action of ACh, adrenaline and substance P, mediated by three separate receptor sites, suggests activation of a common effector mechanism.

Isolated segments of rat parotid gland were mounted in a tissue bath through which oxygenated physiological saline solutions were circulated at 37 °C. Single microelectrodes were used to record both membrane potential and input resistance of surface acinar cells[6]. Substance P (Peninsula Lab.) was applied both by iontophoresis[7,8] and by addition to superfusion media. The iontophoretic application of ACh and adrenaline has been described previously[9].

The mean resting membrane potential was -73.3 ± 0.8 mV ($\pm$s.e.m., $n = 62$) and the mean input resistance of the unstimulated cell was 4.8 ± 0.3 MΩ ($\pm$s.e.m., $n = 38$). Responses to iontophoresis of substance P were recorded in 42 cells from six rats. In 21 of these cells, responses to iontophoresis of ACh were also recorded, and in 12 responses to iontophoresis of three agonists, substance P, ACh and adrenaline, were recorded. Figure 1 shows the similar nature of the responses to the three stimuli in a single cell. Each agonist evokes a marked decrease in input resistance associated with an initial depolarisation. This depolarisation is followed by a delayed hyperpolarisation during which the input resistance returns to the resting level. At less negative resting membrane potentials the initial potential change becomes a hyperpolarisation, but in any one cell the responses to all three stimuli are the same. Such biphasic potential changes have been reported previously in response to iontophoresis of the two autonomic agonists[9]. Only the substance P response was resistant to autonomic blockade by a combination of atropine (10^{-6} M), phentolamine (10^{-5} M) and propranolol (5×10^{-6} M). Responses were recorded in 11 cells and in the presence of all three blockers.

Figure 2 demonstrates the marked reduction in input resistance induced by substance P even in the presence of autonomic blockade. Although there was no detectable change in either the magnitude or duration of the substance P response on introduction of the blockers, the latency of the response was affected. In the control solution the latency of the response was 608 ± 122 ms (mean$\pm$s.e.m. $n = 6$). On introduction of the blockers this latency became $1{,}737 \pm 165$ ms (mean$\pm$s.e.m., $n = 4$),

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‡ To whom correspondence should be addressed.

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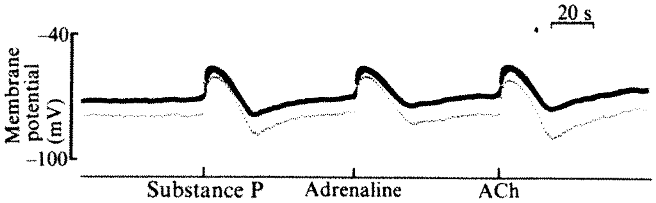

Fig. 1 Recording of membrane potential and input resistance from acinar cell of rat parotid. Responses are seen to iontophoretic application of substance P, adrenaline and ACh. The substance P micropipette contained 3.5 mM of peptide in 20 mM acetic acid (*p*H 5–7). The ejection current was 200 nA applied for 500 ms. Responses were recorded to ejection charges as low as 10 nC. Iontophoresis of a control solution (20 mM sodium acetate acidified to *p*H 5.7) never evoked any response. Adrenaline iontophoresis was achieved at 500 nA for 200 ms; ACh at 100 nA for 1 s. Hyperpolarising current pulses (2 nA, 100 ms) were repetitively injected through the recording microelectrode to obtain input resistance.

suggesting that in the absence of blockade an indirect effect of substance P is manifested at least as a shortened latency. The latency of the direct effects is nevertheless one of the shortest so far recorded for iontophoresis of substance P, variable latencies of between 1 and 30 s having been reported previously[3,7].

Responses could also be evoked by inclusion of substance P in the superfusion media in concentrations of 10^{-7} to 10^{-9} M. Changing the superfusion medium to one containing the drug induced a marked decrease in input resistance, this time associated with a slow monophasic hyperpolarisation. Such responses were obtained in nine cells from eight rats. In five experiments the superfusion medium also contained all three autonomic blockers.

Application of substance P (15 nmol) directly to the tissue bath as a single-shot injection (0.1 ml) evoked responses resembling those induced by iontophoresis. The similar, even repetitive, application of either of two other active polypeptides caerulein or bombesin (0.1 ml containing 1.5 pmol and 3.0 pmol, respectively) failed to evoke a response in any of 12 cells from five preparations. These concentrations are known to be effective in evoking responses from pancreatic acinar cells[10]. This demonstrates that the peptide receptor is not accessible to all active polypeptides and is in agreement with the previous finding[11] that these gastrointestinal hormones cannot evoke salivary secretion.

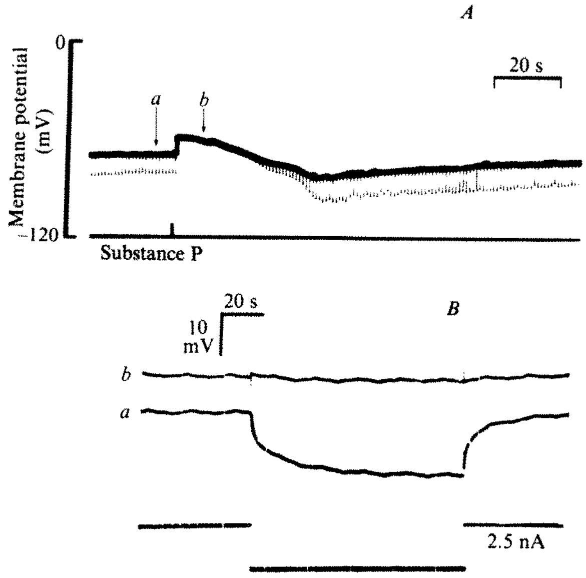

Fig. 2 Substance P response in the presence of full autonomic blockade; that is atropine (10^{-6} M), phentolamine (10^{-5} M) and propranolol (5×10^{-6} M). *a*, Response to iontophoresis of substance P (200 nA for 500 ms); *b*, oscilloscope picture showing amplitudes of the electrotonic potential changes due to injection of current pulses (2.5 nA hyperpolarising, 100 ms) at times *a* and *b* indicated in trace *A*. This demonstrates the marked reduction in input resistance at point *b* as a consequence of the iontophoretic application of substance P. This decrease in input resistance is not due to rectification as the current–voltage relationship of these cells is linear as previously shown in mouse parotid[9].

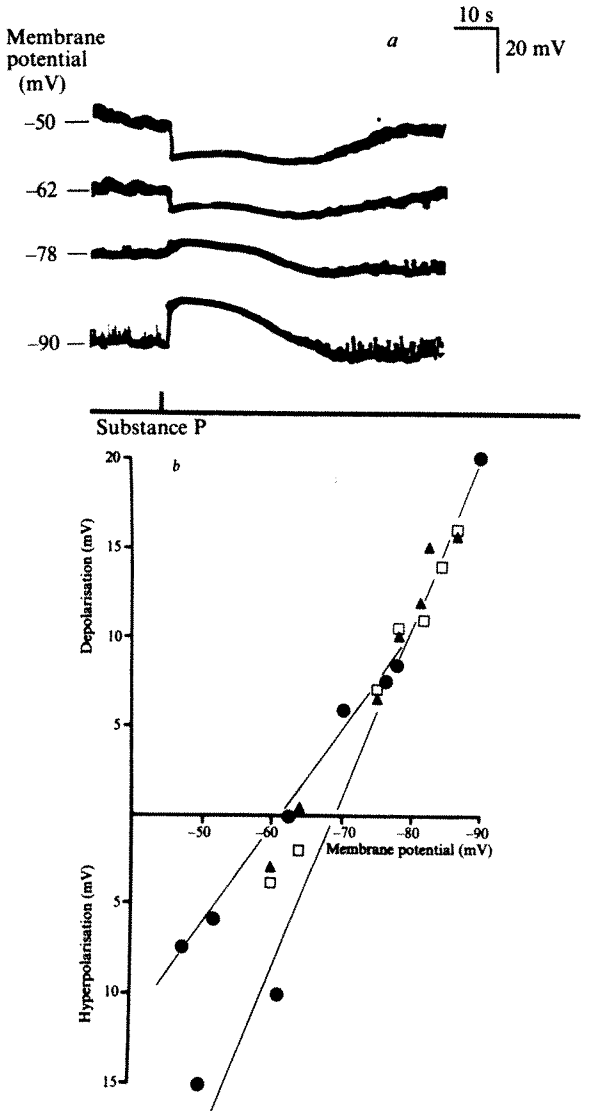

Fig. 3 *a*, Family of traces showing the nature of the substance P response at different levels of membrane potential in the same cell. The membrane potential is varied by applying direct current through the recording electrode. The superfusion medium contained all three autonomic blockers. The response changed from a depolarisation to a hyperpolarisation (reversal) as the membrane potential became less negative. In *b* the amplitudes of substance P responses are plotted as a function of membrane potential (●). Two plots are shown for substance P responses from different preparations and these are compared with plots of ACh (▲) and adrenaline (☐) responses in a third preparation. The reversal potentials of the responses, the intersection with the abscissa, for all three stimuli are between −60 and −70 mV. (Substance P, 100 nA for 1s; ACh, 100 nA for 1s; adrenaline 500 nA for 1 s.)

The reversal potential of the substance P response was determined by two methods. Trautwein and Dudel[12] describe how the 'transmitter equilibrium potential' can be determined by analysis of the changes in both membrane potential and input resistance induced by an agonist. This analysis was carried out for 58 responses to substance P, 35 responses to ACh and 17 responses to adrenaline. The mean reversal potentials were: substance P, −64.4 ± 0.9 mV (±s.e.m.); ACh, −64.5 ± 1.3 mV (±s.e.m.); adrenaline −65.7 ± 2.2 mV (±s.e.m.). The values of the reversal potential for substance P and adrenaline had not been previously determined, but the ACh reversal potential is in very close agreement with the value of −63 mV previously reported[13].

Figure 3 demonstrates the second method used. The amplitude of the responses from a single cell is recorded at different levels of membrane potential. The amplitude of the response is then plotted as a function of membrane potential and the intersection with the abscissa represents the reversal potential. Figure 3*b* shows two plots of responses to substance P and compares them with responses to ACh and adrenaline. Responses to all three stimuli undergo reversal between −60 and −68 mV. Thus both methods of estimating reversal potential give comparable values and illustrate that the reversal potentials for all three stimuli are the same, that is about −65 mV.

Our observations reveal that in a salivary gland substance P evokes changes in membrane potential and input resistance which are resistant to autonomic blockade, probably due to direct activation of a peptide receptor. A degree of specificity of the peptide receptor is indicated by the inability of two other active polypeptides, caerulein and bombesin, to evoke any response. The ionic mechanisms underlying the acetylcholine response has been extensively investigated[13]. The demonstration that substance P and adrenaline share the same reversal potential strongly suggests that responses to the three stimuli, although mediated by separate receptors, are the consequence of activation of the same effector mechanism primarily an increased potassium and sodium conductance.

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Dual action of ototoxic antibiotics on sensory hair cells

Alfons B. A. Kroese & Joep van den Bercken

Institute of Veterinary Pharmacology and Toxicology, University of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands

All aminoglycoside antibiotics in clinical use are ototoxic in that they can cause permanent malfunctioning of the cochlea and vestibule[1–3]. It is generally assumed that these ototoxic antibiotics primarily affect the sensory hair cells in the inner ear and that degeneration of secretory and neural elements comes later[2–5]. Most experimental work on aminoglycoside antibiotic ototoxicity has concentrated on the irreversible effects on inner ear functioning and hair cell morphology[2,3]. Little is known about their mode of action on the hair cells. Interference with hair cell protein synthesis, in analogy with the antimicrobial mechanism, has been ruled out as the primary cause of the high selective[6] toxicity of the aminoglycosides[3,5]. Recent evidence indicates that these antibiotics may interfere with membrane phospholipid metabolism[7]. Morphological studies have demonstrated that early on in the ototoxic process the membrane of the sensory hairs is already damaged[4]. The aminoglycoside antibiotics are reported to have a direct and reversible[8,9] effect on hair cell functioning in organs of the acoustico-lateralis system of lower vertebrates[8–11]. Hence, the primary site of action is probably the hair cell membrane. We have now therefore examined reversible effects of ototoxic antibiotics on single fibre afferent nerve activity and extracellular receptor potentials in the lateral-line organ of *Xenopus laevis.* Our results show that the antibiotics, in concentrations similar to those measured in the cochlear fluids[3], have a dual action on the hair cells. They not only increase the spontaneous afferent nerve activity, probably by an effect on the hair cell membrane, but also severely impair the mechano-electric transduction process, resulting in a large phase lag of the receptor potentials. The latter effect, which is antagonised by Ca^{2+}, may be due to interference of the antibiotics with the mechanical properties of the sensory hairs.

The experiments were carried out on isolated preparations of the lateral-line organ of the clawed frog, *X. laevis.* The organ was stimulated by small-amplitude sinusoidal water movements. Activity of single afferent nerve fibres was computed in period histograms, from which gain and phase were determined as previously described[12]. Amplitude and phase of extracellular receptor potentials were measured as described elsewhere[13]. The effects of the antibiotics were constant within 5 min of application and for concentrations up to 25 μg ml^{-1} were reversible.

Figure 1*a* shows that dihydrostreptomycin in concentrations between 2.5 and 17.5 μg ml^{-1} enhanced spontaneous afferent nerve activity. An increase in spontaneous afferent activity has recently been predicted by Gallais *et al.*[11] from behavioural observations on frogs injected in one ear with streptomycin. It is tempting to conclude that the transient symptom of tinnitus,

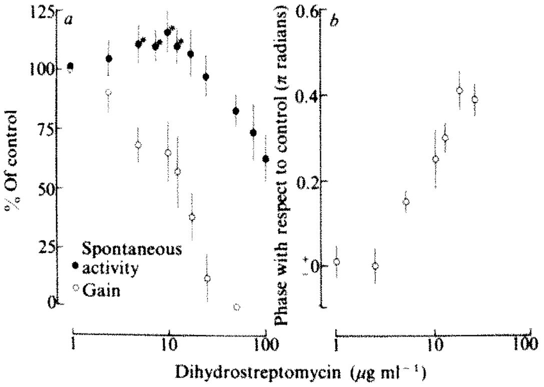

Fig. 1 Effect of different concentrations of dihydrostreptomycin (K & K Laboratories) on spontaneous afferent activity (*a*), and on gain (*a*) and phase (*b*) of the afferent nerve response to stimulation of the lateral-line organ of *Xenopus laevis.* A piece of skin containing several lateral-line organs was placed in an experimental chamber. The inner side of the skin was in contact with Ringer's solution, the outside was covered with tap water, which was replaced every 15 min. Temperature was 18 °C. Each 15-min period with tap water was followed by a 15-min period with fresh tap water to which the antibiotic had been added. Controls were taken before and after each period in which the antibiotic was applied. Stable control values could be obtained from the preparations for a period of more than 2 h. At 7 and 12 min, respectively, after each replacement of the tap water, the lateral-line organ was stimulated for 30–70 s with local, sinusoidal water movements (20 Hz) induced by a small glass sphere. Period histograms of single fibre afferent nerve activity were computed and analysed by means of Fourier analysis. The gain was expressed as per cent modulation of mean afferent activity per μm sphere displacement. The phase of the response was measured with respect to sphere displacement and expressed in radians. Spontaneous activity was measured before each stimulation over 1,000 intervals as the mean number of impulses per s. Further details of recording, stimulation and data analysis can be found elsewhere[12]. The values for spontaneous activity and gain obtained in the presence of dihydrostreptomycin were expressed as a percentage of control. The phase of the response in the presence of dihydrostreptomycin was expressed as phase shift with respect to control. Spontaneous activity was measured in 31 preparations; each point represents the mean ±s.d. of 10 measurements. Gain and phase were measured in 12 preparations; each point represents the mean ±s.d. of six measurements. Asterisk denotes spontaneous activity significantly higher than control (Student's *t*-test; $P < 0.05$)

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which is known to occur soon after administration of ototoxic antibiotics[1], is due to an increase in spontaneous afferent activity of the auditory nerve fibres. Additional experiments showed that blocking of the cholinergic efferent synapse[14] by curare or atropine (5×10^{-5} M) did not prevent the increase in spontaneous activity induced by 10 μg ml^{-1} dihydrostreptomycin. Thus, it is unlikely that the increase in spontaneous activity was brought about by an effect of the antibiotics on the efferent synapse[15]. Apparently, the antibiotics act directly on the hair cell membrane and cause an increase in spontaneous transmitter release[16] from the hair cells.

With higher concentrations of dihydrostreptomycin (>17.5 μg ml^{-1}), spontaneous activity was depressed (Fig. 1*a*). Depression of afferent activity by aminoglycoside antibiotics has been demonstrated previously in the mammalian cochlea[2,3] and in the semicircular canal organ of the frog[9,11]. This effect is consistent with other known effects of high concentrations of aminoglycoside antibiotics, that is, the inhibition of transmitter release[17,18] and postsynaptic blockade[19,16] in the neuromuscular junction, and interference with action potential generation in frog myelinated nerve[20].

The afferent nerve response to stimulation at 20 Hz was markedly depressed by dihydrostreptomycin (Fig. 1*a*). The gain decreased gradually with increasing concentration of the antibiotic and 50 μg ml^{-1} abolished response to stimulation. In addition, dihydrostreptomycin caused a progressive phase lag in the afferent nerve response up to a value of 0.4 π radians at 25 μg ml^{-1} (Fig. 1*b*). Figure 1 clearly illustrates the dual action of dihydrostreptomycin: low concentrations increased the spontaneous activity, but at the same time impaired the mechanical sensitivity.

Other aminoglycoside antibiotics produced a similar dual effect. Streptomycin was about as effective as dihydrostreptomycin (maximum increase in spontaneous activity at 7.5 and 10 μg ml^{-1}, respectively), whereas gentamycin was somewhat less effective (maximum increase at 17.5 μg ml^{-1}). Detailed results will be published elsewhere.

Figure 2 shows that dihydrostreptomycin induced a decrease in amplitude and a phase lag of the receptor potentials almost identical to that measured in the afferent nerve response. A similar phase lag of the receptor potentials was observed at 1 and 5 Hz. The similarity between the effects of dihydrostreptomycin on the afferent nerve response and on receptor potentials demonstrates that the antibiotics primarily affect the hair cells and not the afferent nerve fibres. Further, the antibiotics did not affect the sinusoidal shape of the afferent nerve response[12] or the characteristic dead time in the spontaneous activity.

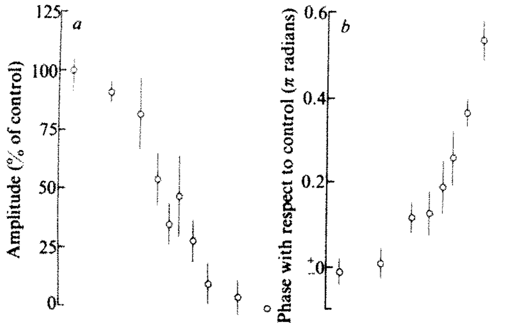

Fig. 2 Effect of different concentrations of dihydrostreptomycin on amplitude (*a*) and phase (*b*) of extracellular receptor potentials recorded from the sensory hair cells in one neuromast of the lateral-line organ of *Xenopus laevis*. Temperature was 22 °C. Preparation and application of the antibiotic were as described in Fig. 1 legend. At 7, 10 and 13 min, respectively, after each tap water replacement, the neuromast was stimulated for 60 s with short sequences of sinusoidal water movements (20 Hz; 8 periods per sequence). Extracellular receptor potentials were recorded by means of Pt–Ir microelectrodes. The response to 30 stimulus sequences was digitised, averaged and plotted. Amplitude and phase with respect to sphere displacement of the extracellular receptor potentials were computed by means of Fourier analysis of the averaged response. Details of recording, stimulation and data analysis can be found elsewhere[13]. Values for amplitude and phase of the response obtained in the presence of dihydrostreptomycin were expressed, respectively, as a percentage of control and as phase shift with respect to control (radians). Results were obtained from nine preparations; each point represents the mean ±s.d. of six measurements.

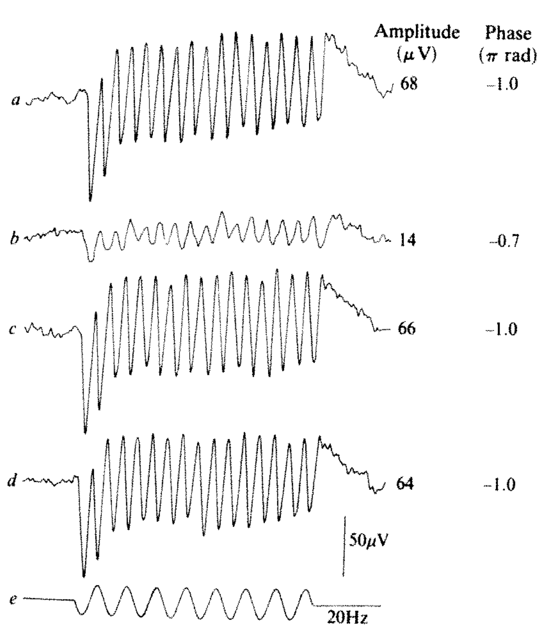

Fig. 3 Prevention of the effect of dihydrostreptomycin on extracellular receptor potentials from the lateral-line organ of *Xenopus laevis* by 5 mM $CaCl_2$. Preparation and application of the antibiotic were as described in Fig. 1 legend. At 13 min after each tap water replacement, the neuromast was stimulated for 60 s with short sequences of sinusoidal water movements (20 Hz; eight periods per sequence) induced by a small glass sphere. Each trace is the averaged response to 30 stimulus sequences. The lowest trace (*e*) denotes the displacement of the glass sphere. *a*, Control; *b*, during application of 17.5 μg ml^{-1} dihydrostreptomycin; *c*, during application of 17.5 μg ml^{-1} dihydrostreptomycin and 5 mM $CaCl_2$; *d*, control. The control recording between *b* and *c* has been omitted. Amplitude and phase with respect to sphere displacement were computed by means of Fourier analysis of the averaged response. To exclude any transient effects, the first two stimulus periods of the averaged response were discarded. Values for amplitude (μV) and phase (radians) are shown on the right side of each trace. Details of recording, stimulation and data analysis can be found elsewhere[13]. The normal Ca^{2+} concentration in the tap water was about 1 mM. Addition of 5 mM $CaCl_2$ alone had no noticeable effect on the receptor potentials.

Aminoglycoside antibiotics are known to interfere with Ca^{2+}-dependent membrane phenomena[7,17,21]. In the present study, the effects of dihydrostreptomycin on receptor potentials were fully prevented by 5 mM $CaCl_2$ (Fig. 3). There is evidence that Ca^{2+} ions are essential for hair cell transduction[22,23], although their precise role remains unclear[23,24]. It has been suggested that Ca^{2+} modulates the permeability of the hair cell membrane to K^+ ions[24], which are thought to carry the receptor current[24,25]. However, the time delay corresponding to the phase lag, particularly at low stimulus frequencies, is too large to be accounted for by an effect on the conductance mechanism underlying the receptor current.

A possible explanation for such a large phase lag is that the antibiotics alter the mechanical properties of the sensory hair bundle. Aminoglycoside antibiotics can cause sensory hair fusion and it has been postulated that they reduce the fixed negative charge on the sensory hair membrane, thereby diminishing the electrostatic repulsion between the hairs[4,26]. The effects reported here were easily reversible, which implies that we have studied an early stage in the ototoxic process. It is conceivable that before sensory hair fusion the antibiotics increase the coupling between the sensory hairs, resulting in a reduced mobility of the hair bundle. An alternative hypothesis is that the antibiotics affect the stiffness of the individual hairs, which depends on their fibrillar core, rather than on their membrane[26]. As the filaments in the core are composed of actin[27], it can be speculated that the antibiotics alter their mechanical properties by interfering with Ca^{2+}. Further work on the action of aminoglycoside antibiotics may lead to a better understanding of the role of Ca^{2+} in the transduction process.

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Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens

J. K. Reddy & D. L. Azarnoff

Department of Pathology, Northwestern University Medical School,
303 East Chicago Avenue, Chicago, Illinois 60611

C. E. Hignite

Research Division, Veterans Administration Hospital,
Kansas City, Missouri 64128

Several drugs, including clofibrate (ethyl-*α*-*p*-chlorophenoxyisobutyrate)[1], are now available for the treatment of hyperlipidaemias[2,3] and others are in the process of preclinical or clinical evaluation[4,5]. Clofibrate, the most widely used hypolipidaemic drug in Europe and the US[2], as well as other potent hypolipidaemic agents, cause massive hepatomegaly when administered to rats[6–8], mice[7,8] or hamsters (J.K.R., unpublished). This hepatomegaly is characteristically associated with a marked increase of peroxisomes (Fig. 1) in the liver cells of all three species[6–8]. These ubiquitous cytoplasmic organelles[9,10] contain catalase, several hydrogen peroxide-generating oxidases, carnitine acetyltransferase[11] as well as enzymes involved in the *β*-oxidation of long-chain fatty acids[12]. The activities of these enzymes in liver are elevated in association with peroxisome proliferation[8,13]. As hepatomegaly and peroxisome proliferation persist for as long as these drugs are administered to the animals, we initiated chronic toxicity studies with selected hepatic peroxisome proliferators in CS^{b} mice and F344 rats. Liver tumours were observed in both rats and mice fed nafenopin (2-methyl-2[*p*-(1,2,3,4-tetrahydro-1-naphthyl)phenoxy]-propionic acid)[14–16] or Wy-14,643 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid)[17], two structurally unrelated compounds (Fig. 2, compounds 2 and 3) which are several times more potent than clofibrate in inducing peroxisome proliferation and hypolipidaemia[8]. Recent evidence indicates that clofibrate (Fig. 2, compound 1) is also carcinogenic when fed to rats at a concentration of 0.5% in the diet[18,19]. We report here that BR-931 ([4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio(*N*-*β*-hydroxyethyl)-acetamide])[20] and tibric acid (2-chloro-5-(3,5-dimethylpiperidinosulphonyl)benzoic acid)[8], two potent hypolipidaemic hepatic peroxisome proliferators (Fig. 2, compounds 4 and 5) induce hepatocellular carcinomas in CS^{b} mice and/or F344 rats. Thus, the development of liver tumours, in animals fed five structually diverse hypolipidaemic compounds (Fig. 2) supports our hypothesis that potent hepatic peroxisome proliferators as a class are carcinogenic.

The cumulative data in Table 1 indicate that BR-931 given at 0.2% dietary level caused a significant increase in the incidence of liver tumours in female CS^{b} mice and male F344 rats. The tumours were multiple and measured 0.5–3.5 cm in diameter. Histologically, these tumours were hepatocellular carcinomas, some of which metastasised to the lungs. BR-931 at 0.05% level induced tumours in 7 of 10 rats; there were few tumours per liver when compared with the liver tumour load in rats fed the higher dose. Tibric acid treatment of rats as outlined in Table 1 also resulted in a significant increase in the incidence of hepatocellular carcinomas. No liver tumours were encountered in the control groups of mice or rats. The ultrastructural appearance of BR-931- and tibric acid-induced hepatocellular carcinomas in rats and mice was characterised by the presence of many peroxisomes in tumour cells, as noted previously in liver tumours induced by nafenopin[14] or Wy-14,643 (ref. 17).

Table 1 Cumulative data on liver tumour incidence in female CS^{b} mice and/or male F344 rats fed hypolipidaemic hepatic peroxisome proliferator BR-931 or tibric acid

Group	Sex	Treatment	No. of animals Initial	No. of animals Effective*	No. of animals with liver tumours (%)	
CS^{b} mice	F	BR-931 (0.2% for 19 months)	15	12	11†	(92)
CS^{b} mice	F	—	15	15	0	(0)
F344 rats	M	BR-931 (0.2% for 16 months)	20	20	20‡	(100)
F344 rats	M	BR-931 (0.05% for 19 months)	10	10	7†	(70)
F344 rats	M	Tibric acid (0.2% for 7.5 months, 0.1% for 4 months and 0.05% for 5 months: total 16.5 months)	35	31	30‡	(97)
F344 rats	M	—	30	30	—	(0)

CS^{b} mice (4–6 weeks of age) derived from a colony maintained in our laboratory (JKR) and F344 rats (weighing 60–90 g) obtained from ARS, Sprague-Dawley, were used. The hypolipidaemic drugs BR-931 (LPB Istituto Farmaceutico) and tibric acid (Pfizer) were added to powdered chow at the concentrations (w/w) shown and fed *ad libitum*. Autopsies were carried out on dead or moribund animals. All surviving animals at the end of the treatment periods shown were killed under light ether anaesthesia.

* The effective number of animals was the number of survivors at the time the first tumour was observed.

† P (one-sided) < 0.001 when compared with the controls by Fisher's exact test.

‡ $P < 0.001$ when compared with the controls by χ^2 test corrected for continuity.

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There was a significant increase in carnitine acetyltransferase activity in the tumours and normal portions of tumour-bearing livers in the BR-931 fed rats, but as expected[18], the activity of peroxisomal marker enzyme catalase was not elevated (data not shown). Similar enzyme changes were previously observed in liver tumours induced by the peroxisome proliferator Wy-14,643 (ref. 17).

These experiments form part of an investigation to test the hypothesis that hypolipidaemic drugs capable of inducing hepatic peroxisome proliferation are carcinogenic. The experiments reported here with BR-931 and tibric acid and previous studies with nafenopin[14,16], Wy-14,643 (ref. 17) and clofibrate[18,19] strongly favour this hypothesis, which can be tested further experimentally because of the availability of several other potent hypolipidaemic agents possessing hepatomegalic and peroxisome proliferative properties. This group comprises structurally diverse chemicals which share unique biological properties, including the ability to induce marked hepatomegaly, hepatic peroxisome proliferation and hypolipidaemia.

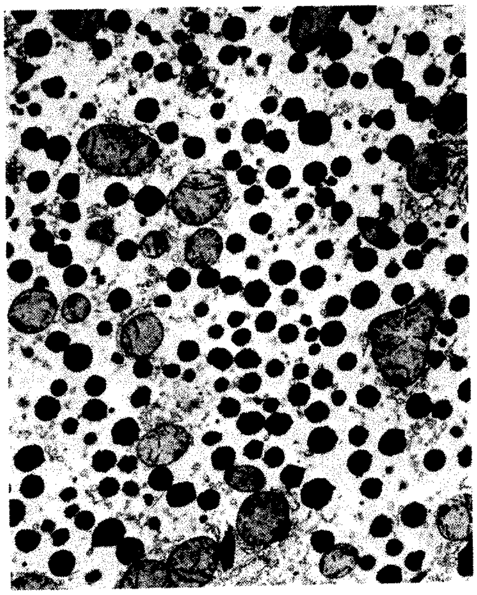

Fig. 1 Treatment with hypolipidaemic drugs causes proliferation of peroxisomes in liver parenchymal cells of rats, mice and hamsters. Many peroxisomes (electron dense bodies) are present in this electron micrograph, which represents a portion of a liver cell of a male rat fed a hypolipidaemic drug at a dietary concentration of 0.1% (w/w) for 4 weeks. The hepatomegaly and peroxisome proliferation persist for as long as the drug is administered. The hypothesis that chemicals capable of inducing hepatic peroxisome proliferation are carcinogenic is supported by the observation that five structurally diverse peroxisome proliferators are carcinogenic in mice and or rats.

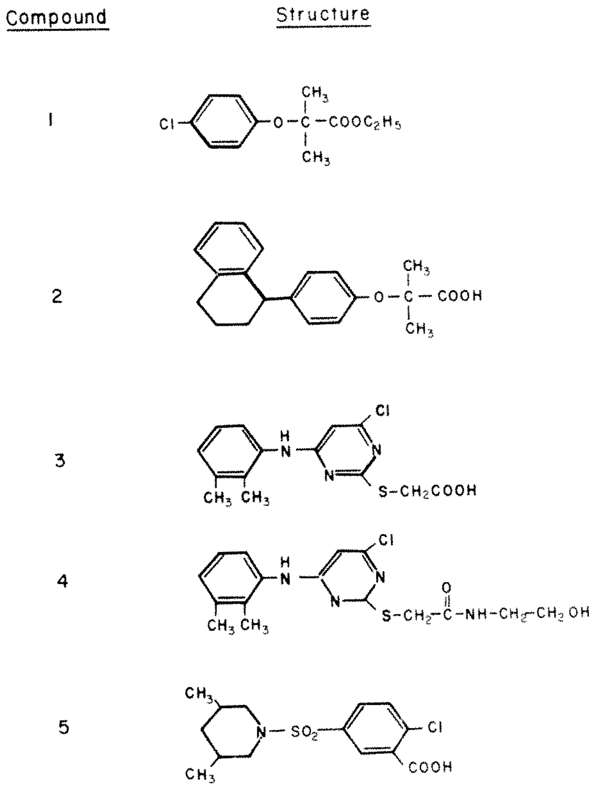

Fig. 2 Chemical structures of selected hypolipidaemic hepatic peroxisome proliferators tested for carcinogenicity. Compound 1: ethyl-α-p-chlorophenoxyisobutyrate (clofibrate, Atromid-S); Compound 2: 2-methyl-2-[p-(1,2,3,4-tetrahydro-1-naphthyl)-phenoxy]propionic acid (nafenopin); Compound 3: [4-chloro-6-(2,3-xylidino)2-pyrimidinylthio]acetic acid (Wy-14,643); Compound 4: [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio(*N*-*B*-hydroxyethyl)-acetamide] (BR-931); Compound 5: 2-chloro-5-(3,5-dimethylpiperidinosulphonyl)benzoic acid (tibric acid). All these compounds induced a significantly high incidence of hepatocellular carcinomas in mice and/or rats.

For these reasons, the hepatic peroxisome proliferators may be considered as a unique class of chemical carcinogens. Furthermore, unlike most known chemical carcinogens which inhibit DNA synthesis[21], the carcinogenic hepatic peroxisome proliferators identified thus far seem to stimulate DNA synthesis and cell division in intact liver without causing any antecedent necrosis[8,17,20]. Finally, none of these compounds caused detectable mutagenic activity in the *Salmonella*/microsome assay, or produced DNA damage in the lymphocyte ^{3}H-thymidine incorporation assay[22]. The mechanism by which these drugs induce liver tumours and the role, if any, of peroxisomal enzymes in, for example, excessive production and/or breakdown of H_2O_2 (ref. 17) or hypolipidaemia, and in the initiation of carcinogenesis such as that invoked for lysosomal DNase in inducing chromosomal aberrations[23], remain to be examined. Whatever the mechanism(s) involved, the morphological finding of hepatic peroxisome proliferation seems a good indication for selecting a potential hypolipidaemic chemical for long-term carcinogenicity testing.

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Viroid synthesis: the question of inhibition by actinomycin D

L. K. Grill & J. S. Semancik

Department of Plant Pathology and Cell Interaction Research Group, University of California, Riverside, California 92521

Viroids, pathogenic, low molecular weight (10^5) single-stranded circular RNA molecules, represent a unique class of minimal infectious agents. Physical characterisation has culminated in the primary sequence of the 359 nucleotides comprising the potato spindle tuber viroid (PSTV)[1]. However, other than the dramatic expression of disease symptoms in plants[2], little is understood of the synthesis and biological activity of the viroid RNA. Molecular hybridisation techniques resulted in the detection of both viroid complementary RNA[3] and DNA[4] sequences. The putative template role of the DNA sequences was reinforced by the reported inhibition of viroid synthesis by actinomycin D[5–7]. However, viroid-specified proteins do not seem to function in replication or pathogenesis, for *in vitro* translation has not been accomplished[8,9]. Furthermore, no AUG initiation triplets have been found in the primary sequence of PSTV or in the hypothetical complementary sequence. Viroid synthesis, therefore, may be characterised as a markedly host-dependent process and consequently potentially very sensitive to inhibition of DNA-directed functions by agents such as actinomycin D. The accumulation of viroid progeny in nuclei[5,10] and nuclear-rich subcellular fractions[11] has provided clues to the mechanism of viroid synthesis. What was reported as a viroid complementary DNA sequence from DNA-rich extracts of *Citrus exocortis* viroid (CEV)-infected tissue[6] has subsequently been positively identified as complementary RNA[3] that may be associated with DNA-rich preparations. With further purification and treatment, no significant levels of viroid-complementary DNA can be detected in diseased *Gynura aurantiaca* or tomato. Therefore, we feel an RNA intermediate must assume a predominant role in any scheme for CEV replication and/or pathogenesis. We now report that synthesis of CEV and PSTV are not inhibited by actinomycin D concentrations which reduce host 5S RNA synthesis by 80%. Thus, the primary role of actinomycin D in viroid synthesis may reflect either inhibition of a host-specified function or a general toxic effect on cell metabolism, and not evidence for the replication through a DNA intermediate.

Because actinomycin D produces toxic conditions in infiltrated tissue[6] as well as protoplast[7] systems, we have used a range of actinomycin D concentrations coupled with the monitoring of host nucleic acid as an internal control to produce a dose–response curve to evaluate actinomycin D sensitivity. Either foliar tissue of *Gynura aurantiaca* infected with CEV or potato tuber sprouts infected with PSTV were vacuum infiltrated with actinomycin D and ^{32}P in a Barth's solution (88 mM NaCl, 1 mM KCl, 0.83 mM $MgSO_4$, 0.41 mM $CaCl_2 \cdot 2H_2O$, 0.34 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2.4 mM $NaHCO_3$, 1.5 mM Tris-HCl, *p*H 7.6) and incubated overnight before the extraction of nucleic acid by the phenol method[12]. Using the parameter of incorporation of ^{32}P into DNA, it is possible to differentiate between sublethal doses of actinomycin D (0–10 μg ml^{-1}) at which DNA synthesis is not affected and higher doses (10–40 μg ml^{-1}) at which the inhibition of DNA synthesis may reflect general cellular impairment (Fig. 1). Incorporation of ^{32}P into total RNA decreased in a linear manner over the entire actinomycin D dose range.

RNA species were analysed by electrophoresis on 5% polyacrylamide vertical slab gel (Figs 2, 3)[13]. Samples were equalised by ^{32}P content so as to monitor only metabolically active cells containing ^{32}P. The intensity of the RNA bands on the autoradiograph was quantitated by direct scanning of the X-ray film at 600 nm.

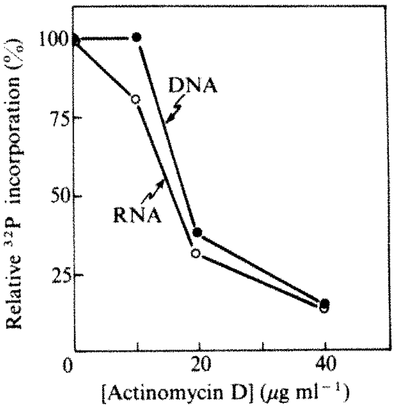

Fig. 1 Relative amounts of ^{32}P incorporation into RNA and DNA as determined by trichloroacetic acid (10% w/v)-precipitable radioactivity using equal nucleic acid concentrations. DNA radioactivity was determined from the acid-precipitable nucleic acids remaining after treatment with RNase A (Worthington, 200 units ml^{-1} at 25°C for 45 min). RNA radioactivity was determined similarly after treatment with DNase I (Worthington, 20 μg ml^{-1} at 25 °C for 15 min).

As purification of the viroid RNA involves partitioning into the 2 M LiCl-soluble fraction, the most appropriate internal control was the 5S ribosomal RNA. The synthesis of this low molecular weight (~36,000) species is significantly inhibited (Fig. 4) over the actinomycin D concentration range. The higher molecular weight ribosomal RNAs purified in the 2 M LiCl precipitate were similarly inhibited by actinomycin D. These results agree with the classical mode of action of actinomycin D as an inhibitor of DNA-directed RNA synthesis.

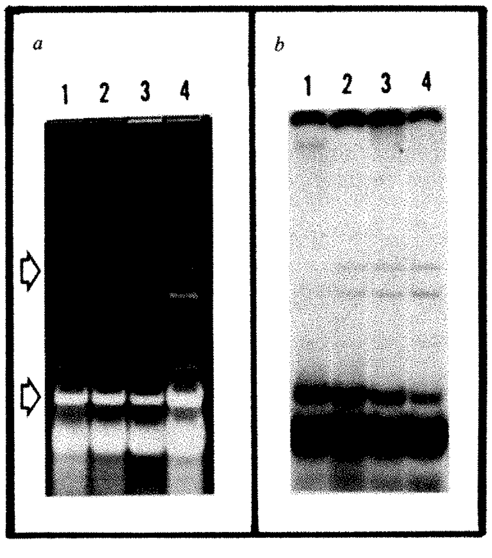

Fig. 2 Polyacrylamide slab gel (5%) electrophoretic patterns of 2 M LiCl-soluble nucleic acids obtained after ^{32}P incorporation into *Gynura* leaf tissue as described in the text. *a*, Nucleic acid patterns after staining with ethidium bromide (0.1 μg ml^{-1}) in healthy tissue (1), and CEV-infected tissue without addition of actinomycin D (2), with 10 μg ml^{-1} actinomycin D (3), or with 20 μg ml^{-1} actinomycin D (4). *b*, Autoradiograph of the same gel after exposing Kodak XR-5 film for 7 days. All nucleic acids were treated with DNase I (Worthington, 2 μg ml^{-1} for 5 min at 25°C) just before loading on to the gel. The arrows indicate positions of CEV RNA (top) and 5S ribosomal RNA (bottom).

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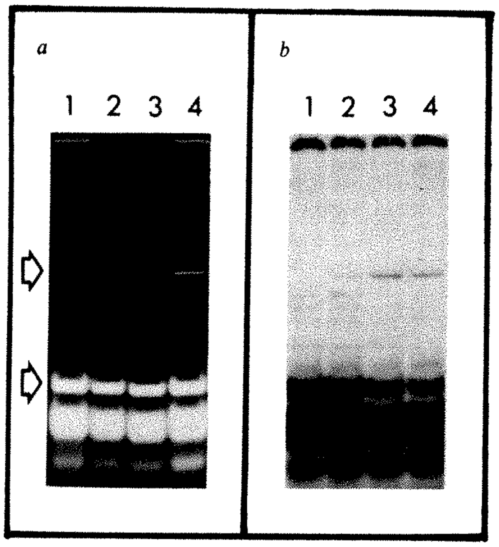

Fig. 3 Polyacrylamide slab gel (5%) electrophoretic patterns of 2 M LiCl-soluble nucleic acids obtained after ^{32}P incorporation into potato sprouts as described in the text. *a*, Nucleic acid patterns after staining with ethidium bromide in healthy potato sprouts (1), PSTV-infected potato sprouts without addition of actinomycin D (2), with 10 μg ml^{-1} actinomycin D (3), or with 20 μg ml^{-1} actinomycin D (4). *b*, Autoradiograph of the same gel. The nucleic acids were treated with DNase I as described in Fig. 2 legend. The arrows indicate positions of PSTV RNA (top) and 5S ribosomal RNA (bottom).

In contrast to the inhibition of host RNA synthesis, the synthesis of CEV in *Gynura aurantiaca* and potato spindle tuber viroid in potato (Figs 2, 3) was not as affected by actinomycin D. Therefore, it is not possible to confirm the previously reported actinomycin D inhibition of PSTV[5,6] and cucumber pale fruit viroid[7] in tomato. Because, in these systems, relatively high concentrations of actinomycin D were used to reduce total RNA synthesis by 80–90%, the interpreted inhibition may have resulted from a general toxic effect on cell metabolism and not the specific action of actinomycin D. Furthermore, the autoradiography procedure used here for analysing the synthetic rate of RNA species is more sensitive than slicing of cylindrical gels.

The absence of any significant inhibition of viroid synthesis is consistent with the primary role of the CEV complementary RNA sequences as intermediates in viroid replication[3]. Any inhibition of viroid replication by actinomycin D would seem to be mediated through a host-genome specified function and not through a viroid complementary DNA sequence. This inhibition by actinomycin D may be analogous to the sensitivity of viral RNA-directed RNA synthesis during early phases of infection[14]. Complementary RNA sequences in the PSTV system have been detected, whereas confirmation for the existence of complementary DNA sequences could not be accomplished (M. Zaitlin, unpublished). These data are consistent with the present finding that viroid synthesis is not specifically inhibited by actinomycin D. Data suggesting the absence of inhibition by actinomycin D have been confirmed by T. J. Morris (unpublished) using a different technical approach with the PSTV system.

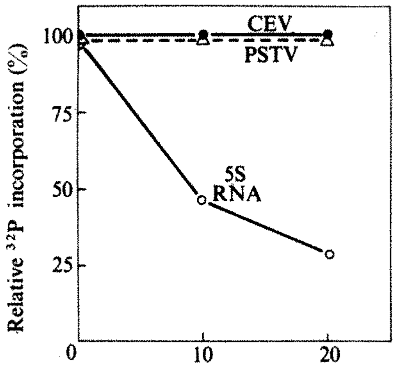

Fig. 4 Incorporation of ^{32}P into viroid RNA (CEV, PSTV) and 5S ribosomal RNA as influenced by actinomycin D concentration. The intensity of the radioactive RNA bands observed in the autoradiograph of the 5% polyacrylamide gel were quantitated by scanning the film at 600 nm and determining the area of each peak. The concentration of the radioactive viroid RNAs were set at 100% and the radioactive 5S RNA concentration determined accordingly.

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Immune and virus-induced interferons may activate cells by different derepressional mechanisms

F. Dianzani, M. Zucca, A. Scupham & J. A. Georgiades

Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas 77550

Virus-induced interferon (VIF), and immune interferon (IIF) produced by lymphocytes following activation by various mitogens (phytohaemagglutinin, concanavalin A, staphylococcal enterotoxin A) or by specific antigenic stimulation (tuberculin, bacterial toxoids, and viral antigens) show important functional differences. These include different antitumour[1] and immunoregulatory activities[2] and differential inhibition of activity by mercaptoethanol[3]. Another important difference has recently been shown: cells treated with IIF acquire the antiviral state much more slowly than those treated with VIF[4]. This may be due to (1) difference in cellular receptors, (2) presence of an inhibitor of antiviral activity in the IIF preparations, or (3) a different mechanism of activation of the antiviral state by the two types of interferon. The first two hypotheses seem unlikely, since (1) no difference between cell association by the two types of interferon has been detected[4], and (2) the presence of the slow acting IIF preparation did not inhibit the rapid activation by VIF[4]. The present study shows that the different kinetics of induction of the antiviral state result from a major difference in the mechanism by which IIF and VIF activate cells.

It has generally been accepted that interferon itself is not the direct inhibitor of viral replication[5]. Instead, interferon acts by inducing the cells to produce one or more secondary protein(s), the so called antiviral or effector protein(s), which are needed for ultimate antiviral activity by cells. In support of this model one laboratory has reported that at least one newly formed protein correlates with the antiviral activity of VIF-treated cells[6]. It has also been shown, by using metabolic inhibitors, that VIF directly induces the production of the antiviral protein(s) without first

Table 1 Effect of cycloheximide on development of the antiviral state by virus-type and immune interferons

Type of interferon*	Cells	Cycloheximide added	$\log_{10}$ virus HA yield†
None	Mouse L	No	2.1
		Yes	2.1
Mouse, virus-induced	Mouse L	No	<0.3
		Yes	<0.3
Mouse, immune	Mouse L	No	<0.3
		Yes	1.5
None	Human WISH	No	1.8
		Yes	1.8
Human, virus-induced	Human WISH	No	<0.3
		Yes	<0.3
Human, immune	Human WISH	No	<0.3
		Yes	1.5
None	Human foreskin	No	1.8
		Yes	1.8
Human, virus-induced	Human foreskin	No	<0.3
		Yes	<0.3
Human, immune	Human foreskin	No	<0.3
		Yes	1.8

Mouse virus-induced interferon was obtained from mouse C-243 cell cultures induced with Newcastle disease virus as previously described[9]. Human virus-induced interferon was obtained by the Antiviral Substances Program, NIAID and was produced as described previously[10]. Mouse and human immune IFs were obtained by stimulation of lymphoid cell cultures with staphylococcal enterotoxin A and were characterised as immune-type IFs as described previously[11].

* Interferon used at 100 reference units ml^{-1}.

† GD-7 virus haemagglutinin yield was used for measurement of resistance in mouse cells, and Sindbis virus haemagglutinin yield was used for human cells.

inducing intermediary proteins[7]. Specifically, mammalian cells treated with VIF in the presence of cycloheximide developed the antiviral state immediately after reversal of the drug action. This finding indicates that an mRNA leading to the production of the antiviral protein was transcribed in the presence of VIF and cycloheximide and was translated only after reversal of cycloheximide action[7]. The addition of actinomycin D before removal of cycloheximide did not block the development of viral resistance. This suggests that VIF directly induces the antiviral protein without the need of intermediary proteins.

The same experimental plan was used to study the mechanism of activation of the antiviral state by IIF. In a first set of experiments, cultures of murine or human cells were treated with cycloheximide and then exposed to 100 reference units of homologous VIF or IIF, as previously described[8]. In our assay the titres reported as reference units correspond exactly to the actual titres observed in the experiments after 24 h incubation of interferon with the cells. The cultures were washed free of interferon and cycloheximide 4–5 h later, and challenged with GD-7 virus (mouse cells) or Sindbis virus (human cells). The virus yield was measured after 18 h incubation. The results are shown in Table 1.

As has been shown previously, cycloheximide did not prevent development of the antiviral state in mouse or human cells treated with VIF, suggesting that the mRNA for the effector protein(s) was induced by VIF during inhibition of protein synthesis and was rapidly translated after removal of cycloheximide. In contrast, cells treated with human or mouse IIF in the presence of cycloheximide failed to develop a significant level of antiviral activity. This finding might indicate that cells treated with IIF in the presence of cycloheximide either did not produce mRNA for the effector protein(s) or else they did produce this mRNA for the antiviral protein(s) but it was not translated quickly enough to prevent replication of the challenge viruses. To test these hypotheses, two-stage experiments were designed. During the first stage, production of mRNAs was permitted to occur by treating cells with VIF or IIF during inhibition of protein synthesis by cycloheximide, while in the second stage these mRNAs were allowed to be translated after addition of actinomycin D to block further RNA transcription. Specifically, cell cultures were pretreated with cycloheximide for 15 min and then treated with VIF of IIF in the continued presence of cycloheximide. After 3 or 5 h actinomycin D was added to some of the cultures to inhibit any further transcription of mRNA and 30 min later the cultures were washed three times (to remove interferon and reverse the inhibition of protein synthesis by cycloheximide but not the inhibition of transcription of RNA by actinomycin D). Some of the cultures were then challenged immediately, while others were challenged 1 and 3 h later. The results of a representative experiment are shown in Fig. 1.

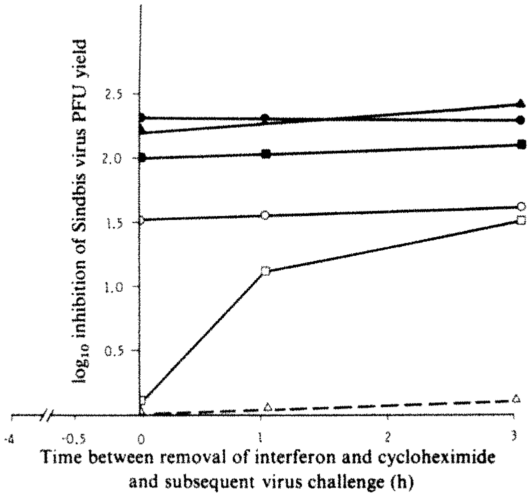

Fig. 1 Development of the antiviral state in human foreskin fibroblast cultures challenged at various times after exposure to human leukocyte interferon (VIF) or immune interferon (IIF) in the presence or absence of metabolic inhibition of protein or RNA synthesis. Four sets of cultures (six tubes per set) were pretreated with cycloheximide, 20 $\mu\text{g ml}^{-1}$, and 15 min later, 100 reference units of VIF (■) or IIF (□) were added to each tube. At the same time VIF (●) or IIF (○) was added to other cultures not treated with cycloheximide. After 3.5 h of incubation at 37 °C, actinomycin D, 5 $\mu\text{g ml}^{-1}$, was added to two of the sets of cultures treated with cycloheximide and IF (▲ and △). After 30 min, IF and the inhibitors were removed by multiple washing and the cultures were challenged thereafter at the indicated times with Sindbis virus. The level of the antiviral state was measured by determining the inhibition of virus yield in a single growth cycle as described previously[9]. Control cultures treated with the metabolic inhibitors as indicated above showed that protein synthesis (as measured by incorporation of ^{14}C-leucine) was inhibited by 95% but RNA synthesis (as measured by incorporation of ^{3}H-uridine) was unaffected during treatment with cycloheximide. After removal of cycloheximide, protein synthesis resumed fully within 0.5 h, indicating the reversibility of the inhibitor. In comparison, transcription was irreversably blocked (92%) by the added actinomycin D. Additional control experiments showed that: (1) cell cultures treated with metabolic inhibitors for the times indicated above gave full yield of virus after removal of the drugs, and (2) treatment of the cells with 5 $\mu\text{g ml}^{-1}$ of actinomycin D for 30 min before addition of IF was completely able to prevent the development of the antiviral state. During the experiments the incubation temperature was carefully maintained at 37 °C as described previously[8].

It may be seen that, as previously observed[7], cultures treated with VIF developed maximal resistance when challenged immediately after removal of interferon, regardless of the presence of cycloheximide and the subsequent addition of actinomycin D. This result confirms the direct induction of the effector protein(s) by VIF. In contrast, cultures treated with IIF in the presence of cycloheximide alone failed to show detectable resistance when challenged with virus immediately after removal of the inhibitor, but gradually acquired the antiviral state when challenged 1 and 3 h later. The somewhat lower level of antiviral activity which appears to be induced by IIF as compared to VIF in Fig. 1 is due to the slower kinetics of cell activation by IIF (ref. 4) since the same activity was induced by both IFs at 24 h. However, again in contrast to the results with VIF, if actinomycin D was added before removal of cycloheximide, no detectable resistance appeared. The same results were obtained in mouse L cells treated with mouse VIF or IIF or when 30 μg per ml of cordycepin or 1-β-ribofuranosylbenzimidazole (DRB) were used as inhibitors of mRNA synthesis instead of actinomycin D, or when larger amounts of interferon (500 units instead of 100) were used. These findings rule out the delayed translation hypothesis and suggest that in the presence of cycloheximide IIF does not directly induce the formation of mRNA for the effector protein(s).

To further exclude the possibility that the IIF-induced mRNA for the antiviral protein(s) is translated more slowly than that induced by VIF, mRNA was induced in cells treated with IIF alone. Its rate of translation was then determined by blocking further mRNA synthesis with actinomycin D and thereafter measuring the level of viral resistance every hour. The data showed that cells stimulated with IIF, treated with actinomycin D after 3.5 h, and then challenged immediately or every hour thereafter for 4 h developed 1.5 $\log_{10}$ of viral inhibition prior to the addition of actinomycin D; however the level of resistance did not rise thereafter, indicating that, similar to VIF, there is no delayed translation of the mRNA for the effector protein(s).

Another, though unlikely, possibility is that IIF simply induces a less stable mRNA for the antiviral protein. If this were the case, then the development of resistance in cells which would have produced the putative unstable mRNA after treatment with IIF and cycloheximide would begin at the same time as in cells containing stable, VIF-induced, mRNA but the maximum level of resistance would be lower. Instead, the first development of resistance is late and the maximum resistance is the same (Fig. 1). There are other more complex possible explanations, considering the multiple variables, but it is clear that IIF represents a novel mechanism, requiring a more complex cell-activating process than does VIF.

Taken together, the findings imply that there are multiple genetic derepressional events in the mechanism by which IIF activates the antiviral state. This interpretation is necessarily indirect due to the use of metabolic inhibitors as probes; however the conclusion is relatively strongly supported since the specificity of action of actinomycin D, cordycepin and DRB has been reasonably well established. Therefore the most probable interpretations are that VIF directly induces a mRNA for the antiviral or effector protein(s)[7], but IIF does not directly induce functional mRNA for the effector protein(s). Studies are in progress to identify the product(s) of the gene(s) activated by IIF.

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Thymic nurse cells—Ia-bearing epithelium involved in T-lymphocyte differentiation?

Hartmut Wekerle & Uwe-Peter Ketelsen

Max-Planck-Institut für Immunbiologie, Freiburg, FRG

The vertebrate thymus has a crucial role in the development and organisation of the immune system. Physiologically, maturation of primitive incompetent precursor cells to immunocompetent T lymphocytes takes place in the thymus[1]. Recently, evidence has been presented suggesting that the capacity to recognise determinants of the self major histocompatibility complex (MHC), which underlies MHC restriction of T-lymphocyte recognition, is also acquired within the thymus[2]. Pathologically, T-cell lymphomas are known to arise within the thymus[3]. In all three cases, it is probable that at least some developmental events require close spatial interactions, if not direct contact, between migrating lymphoid cells and sessile stromal thymus elements. We describe here a state of maximal contact between both thymic cell partners, which might represent certain contact-dependent stages of T-cell development. We found that in all normal mouse and rat strains tested so far, the thymuses contain a sizeable number of huge cells of epithelial origin, which engulf and release up to 50 thymic lymphocytes. As the epithelial cells do not degrade the invading lymphocytes, but may rather provide microenvironmental requirements necessary for lymphocyte proliferation and differentiation, we call them 'thymic nurse cells' (TNCs). These TNCs express all the MHC determinants required by a cell involved in the establishment of T-lymphocyte MHC restriction. They express high doses of K/D antigens of the H–2 complex, and equally high doses of I–A region determinants. However, TNCs lack surface immunoglobulin and T-lymphocyte-specific Thy-1 antigen.

We have already developed a method that allows culture of rodent thymus stromal cells as pure monolayers[4]. This method is based on tryptic dissociation of minced thymic tissue by a stepwise digestion procedure. After repeated, exhaustive trypsinisation of thymic material, we recently found that unusually large, intact round cells (diameters up to 50 μm) can be recovered from all preparations. These round cells, TNCs, contain high numbers of small to medium-sized lymphoid thymocytes. Due to the enormous size of these cells, it is possible to isolate them from other, smaller lymphoid or stromal elements by simple repeated sedimentation through fetal calf serum layers. We recover routinely about 15×10^3 TNCs from one young adult mouse thymus and up to 10×10^4 TNCs from corresponding rat organs.

A number of observations suggest that TNCs are epithelial cells rather than macrophages, and that the engulfed lymphocytes have actively entered the TNCs rather than being phagocytosed. The crucial morphological features of TNCs revealing their epithelial nature are demonstrated in Fig. 1. This ultra-thin section shows a typical TNC incorporating at least seven thymocytes. The well organised tonofilament bundles in the TNC cytoplasm, and the typical vacuolisations make them indistinguishable from the cortical epithelial cells described *in situ*[5]. TNCs also functionally behave like epithelial cells. They are released from the thymic stroma only after extensive trypsinisation. They thus must be firmly anchored within the stromal network. This is in contrast to macrophages, which can be released by mere mechanical dissociation. When taken into culture, TNCs do not promptly adhere to plastic surfaces, as macrophages do, but rather tend to form cell aggregates. Finally, long-term cultures of purified TNC fractions display classical epithelial morphology lacking both mesenchymal and macrophage components, which may predominate in infractionated thymus cultures.

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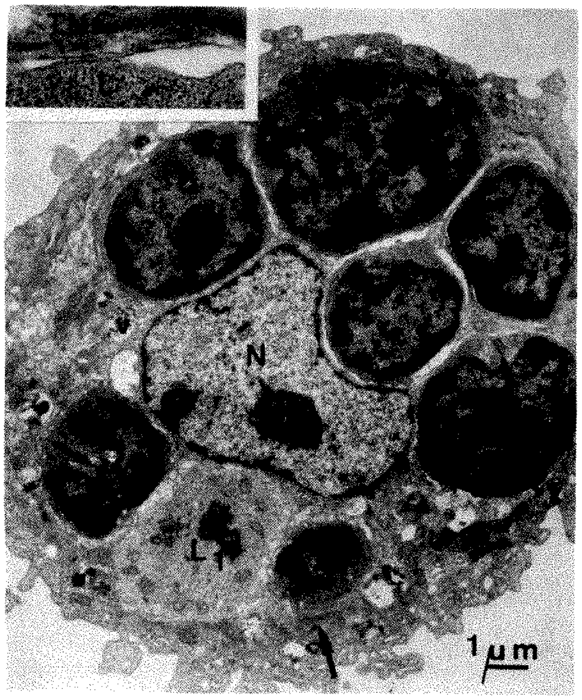

Fig. 1 Thymic nurse cell containing intact, proliferating thymocytes. Mitotic thymocyte (L_1), and centrioles in thymocyte cytoplasms (arrows). TNC nucleus (N) with typical prominent, reticular nucleolus. Prominent features of the TNC cytoplasm: vacuoles filled with coarse, granular material (v), and tonofilament streams in the periphery (double arrow). Inset: higher magnification of contact area between thymic lymphocyte (L) and TNC-containing tonofilaments. Thymuses from 40 young adult female C3H/f mice were washed in cold phosphate-buffered saline (PBS), minced with scissors, and subjected to differential trypsinisation[4]. The minced tissue was transferred into a trypsinisation bottle, and agitated in cold PBS for 5 min to remove most of the loose thymocytes and macrophages. The remaining tissue fragments were softened by further agitation in warm trypsin/DNase solution (50 ml 0.25% trypsin, plus 0.02 mg ml^{-1} DNase in Ca^{2+}- and Mg^{2+}-free PBS) for 10 min. After discarding the supernatants, the main trypsinisation rounds were carried out in warm trypsin/DNase solution for 20 min per round. Single cell supernatants containing appreciable numbers of TNCs were pooled and resuspended in 3 ml HEPES-buffered Eagle's medium (EM-H). This suspension was layered on top of 10 ml undiluted, heat-inactivated fetal calf serum (FCS) in a conical 40-ml glass centrifuge tube. After sedimentation at unit gravity for 15 min, the top fraction containing the majority of all small cells was discarded, whereas the serum fraction and the sediment containing the large TNCs were collected. The cells were resuspended in 0.5 ml EM-H, layered over 2 ml FCS in a 10-ml Falcon plastic tube, to purify further the TNC populations. Practically pure TNC fractions were obtained after 4 or 5 sedimentation rounds. For electron microscopy TNC fractions were fixed in 3% glutaraldehyde, postfixed in 1% OsO_4, embedded in Epon before ultra-thin sections

The majority of the thymocytes engulfed in TNCs appear to be fully intact, as judged by morphological criteria. Many of these thymocytes seem to be involved in proliferation, as suggested by the unusually high incidence of mitotic figures and centrioles (Fig. 1). The thymocytes are completely enclosed by a TNC membrane, but we never observed signs of fusion between both cell partners. That the surrounding caveolar cleft does not communicate with the extracellular space is indicated by the finding that antibodies binding to thymocytes, for example anti-Thy-1 antibodies, do not reach the engulfed thymocytes. Our observation that initially pure TNC fractions cultured overnight contain high numbers of viable free thymocytes suggests that at least a major proportion of the internalised thymocytes can migrate out of the TNCs.

The phenomenon that migratory cells can invade larger, sessile cells without being digested, has been termed 'emperipolesis'[6], and was mainly described in tissue culture conditions. Note, however, that *in situ* emperipolesis of thymocytes in thymic stroma cells has been observed in normal rat thymuses[7], as well as in thymuses from mice undergoing graft-versus-host disease[8]. It seems reasonable to assume that migration of thymocytes into autochthonous stromal cells is initiated by recognition of stromal determinants by the migrating thymocytes. Indeed it has been shown[9] that normal thymocyte populations contain considerable numbers of clones capable of recognising self determinants expressed on autochthonous stroma cells.

It is tempting to speculate that the TNC phenomenon has some role in intrathymic T-lymphocyte differentiation, and, in particular, in the T-cell education/selection process, as recently proposed by Zinkernagel[10] and Bevan[11]. These authors found that the capacity of the T-cell compartment to acquire self-MHC-restricted reactivity against foreign determinants, which may coincide with generation of T-cell diversity[12], is developed within the thymus. T-lymphocyte reactivity is known to be restricted by self determinants coded for both by K/D as well as I regions of the murine MHC. If selection processes within the thymus would involve TNCs, both K/D and I region determinants should be expressed on TNC membranes.

We determined the H–2 antigen formula of TNC membranes using indirect immunofluorescence and monoclonal antibodies, which are directed against determinants of various H–2 subregions (Table 1). TNCs which do not bind anti-Ig and anti-Thy-1.2 antibodies express high doses of classical K/D region histocompatibility antigens on their surface, and equally high concentrations of I–A region determinants. The latter finding is particularly remarkable, as I region antigens are known to be restricted to relatively few cell types, such as (B) lymphocytes, macrophages and sperms[13].

Some recent reports have demonstrated the presence of I-region antigens on thymic components. However, it has proved difficult to classify exactly the antigen-positive cell type using light microscopical screening of thymus sections[14,15]. This holds true in particular for distinction between thymus epithelium and thymus macrophages, which may be abundant constituents of the organ[16]. We applied a fluorescence double labelling method, which allowed functional classification of macrophages, and, at the same time determination of the surface antigen phenotype. Cellular phagocytosis was determined using TRITC-labelled zymosan, whereas the membrane antigens were assayed by indirect immunofluorescence using monoclonal antibodies and FITC-RAMIG, as described in Table 1. Figure 2 shows a mixed T-cell population composed of TNCs, macrophages and a few thymocytes, which were first exposed to

Table 1 Antigens expressed on TNC membranes

Antisera (batch code)*	C3H/f (H–2^k) TNC†	C3H/f (H–2^k) Thymocytes	C57BL/6 (H–2^b) TNC	C57BL/6 (H–2^b) Thymocytes
FITCS–RAMIG alone	–	–	–	–
+ anti-Thy-1.2 (F7D5)	–	+ +	–	+ +
+ anti-K^k (H100-27R9)	+ +	±	±	–
+ anti-I–A^k (H118-49)	+ +	–	±	–
+ anti-I–A^b (B17-123.R4)	±	–	+ +	–
+ anti-H–2D^b (B22-277.R1)	–	–	+ +	±

* Monoclonal hybridoma antibodies were derived from hybridoma-induced ascitic fluid[17]. Anti-Thy-1.2 (F7D5) was from Olac. FITC–RAMIG was a fluorescein-labelled rabbit anti-mouse immunoglobulin antibody preparation from Miles–Yeda.

† Cell preparation and labelling procedures as in Fig. 1 legend. The mice were 4–6-week-old females. Fluorescence was scored: + + bright, distinct ring-shaped fluorescence; + definite, though less intensive ring shaped; ± vague fluorescence, without ring formation, and – no fluorescence at all.

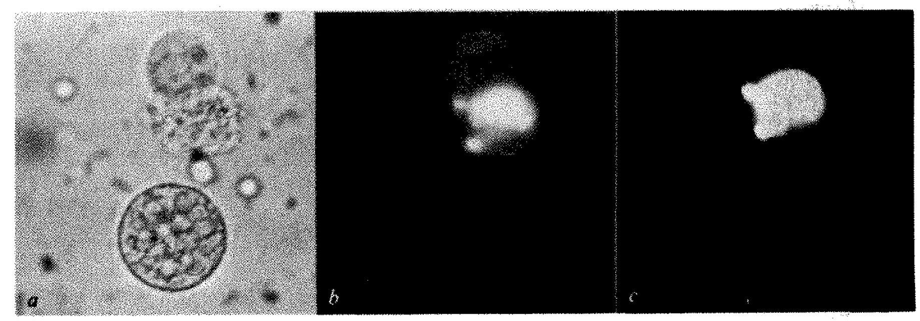

Fig. 2 Expression of Ia antigens on TNCs and thymic macrophages. *a*, Light microscopy of a semi-purified TNC fraction prepared from C3H/f thymuses following the methods described in Fig. 1. TNC (lower cell), thymic macrophage (centre), and unclassified thymic stromal cell (top), along with three thymocytes. *c*, TRITC-labelled zymosan ingested by a thymic macrophage, viewed under red-pass filter. Zymosan (20 mg, lot no. DZ-3185, Schwarz/Mann) was suspended in 30 ml boiling 0.5 M sodium bicarbonate solution (*p*H 9.5). Total de-aggregation of the particles was achieved by additional short ultrasonication. After cooling the suspension, we added 1.6 mg tetramethyl-rhodamine-isothiocyanate, isomer R (TRITC, Nordic Laboratories). The mixture was stirred overnight at 4 °C. The stained zymosan particles were centrifuged (2,000*g*, 10 min, 4 °C) and washed five times in each 30 ml ice cold acetone water (3 parts distilled water, 1 part acetone), before being resuspended in 1 ml 0.5 sodium bicarbonate solution. This stock solution was stored in the dark at 4 °C. For the phagocytosis assay, TNC populations were resuspended in 1 ml EM-H, supplemented with 10% FCS (1×10^6 cells ml^{-1}). We added 20 μl TRITC–zymosan stock solution. After 30 min incubation at 37 °C in normal atmosphere, the cells were centrifuged and resuspended in 0.5 ml EM-H, and underlayered with 2 ml FCS. Within 15 min, most of the living thymus cells sedimented to the bottom, whereas the small free zymosan particles were left in the upper layer. *b*, Membrane fluorescence of the same cells under green-pass filter. For immunofluorescence demonstration of Ia antigens, TNC populations were resuspended in Eppendorf conical plastic tubes, in 100 μl PBS (plus 0.2% NaN_3). We added 10 μl undiluted monoclonal antibody containing ascites fluid H-118-49 (reacting against Ia.2, I–A^k, ref. 17). After 30 min incubation on ice, the cells were washed twice in each 1 ml PBS, resuspended in 200 μl PBS. After addition of 20 μl fluorescein-labelled rabbit anti-mouse immunoglobulin antibody solution (diluted 1:6 in PBS, Miles–Yeda), cells were incubated on ice for a further 30 min, washed twice, and screened under a Zeiss fluorescence microscope. Photomicrographs were taken on Kodak Tri-X pan film (400 ASA), with an exposure of 60 min. The macrophage ingested zymosan shows some residual fluorescence in these conditions.

TRITC-Zy and subsequently to anti-I-A antibody and FITC-RAMIG. Both macrophages and TNCs, but no free thymocytes, express I-A region determinants, as indicated by the fluorescence under a green-pass filter (Fig. 2*b*). Our investigations showed, moreover, that TRITC–zymosan was never ingested by TNCs, and also that TRITC–zymosan ingesting cells, macrophages, never contained more than one morphologically intact lymphocyte (Fig. 2*c*). This provides further support for our previous ultrastructural evidence that TNCs and macrophages are distinct cell types.

Thus, TNCs indeed possess all antigenic requirements needed by a thymic stromal cell involved in a selection process of self-restricted T-lymphocyte clones. We are attracted by the possibility that immature T-cell progenitors with receptors for self antigens recognise self-MHC determinants on TNC membranes as a first step of selection. The recognising cells could be caused to invade the TNC, and, within the peculiar intracellular environment, could be induced to differentiate to immunocompetent T cells. The unusually high rate of proliferative activity of the engulfed thymocytes seems to support such a hypothesis.

Our data at this stage are merely descriptive and do not relate to actual functions of TNCs in T-cell maturation. Our techniques to isolate practically pure TNC populations from normal thymuses might, however, form a good basis for future experiments.

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Production of alloreactive T-cell lymphomas

C. G. Fathman* & I. L. Weissman†

* Department of Immunology, Mayo Clinic, Rochester, Minnesota 55901
† Laboratory of Experimental Oncology, Stanford University Medical School, Stanford, California 94305

Experiments predicting[1,2] or demonstrating lymphomagenesis in mice undergoing graft-versus-host (G-v-H) or chronic immunological reactions have been reported[3–5]. In such cases, lymphomagenesis seemed to operate through the agency of activated murine leukaemia viruses (MuLV) and occurred only after very long latent periods, and most (but not all) lymphomas were of host origin[4]. No attempts were made in these studies to assess the relative role of T-cell subpopulations in lymphomagenesis. We have proposed elsewhere that certain MuLV-induced T lymphomas might be the progeny of that subset of normal thymocytes bearing antigen specific T-cell receptors for MuLV envelope antigens[6–10]. Further, the MuLV binding to these cells provides both a portal of infection and a mitogenic signal[6,9,10]. These cells and their thymic progeny would be subject to continuous restimulation by the endogenously produced MuLV envelope glycoproteins, and thus the apparent and 'uncontrolled' proliferation of the cells would be due to their inability to resolve the viral infection and escape the resultant antigenic microenvironment. This receptor-mediated leukaemogenesis hypothesis predicts that any T-cell clone that cannot escape its stimulating antigens would appear to be lymphomagenic in that environment. Recent experiments in our laboratory have demonstrated that unique clones of strain A alloreactive T cells exist which can recognise hybrid mixed lymphocyte reaction (MLR)-stimulating determinants present on (C57B16×A/J)F$_1$ (B6A) stimulator spleen cells[11–15]. A variety of such clones have been isolated and characterised[13–15]. We reasoned that, using these clones, it might be possible to generate alloreactive T-cell lymphomas by *in vivo* injection of these cells into hybrid B6A mice. We report our successful results here.

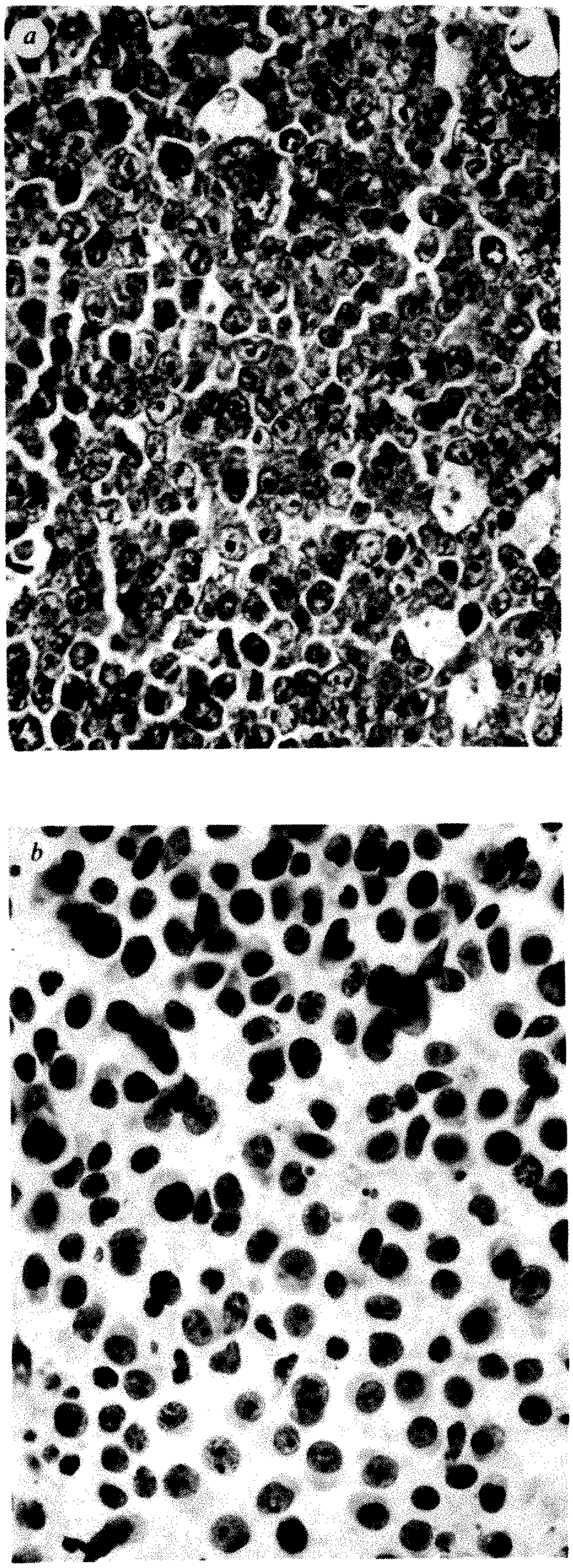

Fig. 1 *a*, Light microscopic appearance of immunoblastic lymphoma involving pancreatic lymph node (initial tumour). *b*, Cellular imprint preparation from pancreatic lymph node demonstrating immunoblastic morphological features of tumour cells: large central nucleoli, thick nuclear membranes and strongly staining expanse of cytoplasm (alcohol fixation, haematoxylin–eosin stain, ×640).

Table 1 Proliferative responses of clone A(B6A)1-1

Stimulator cells				
A	B6	B6A	DBA/2	SJL
2,105 ±198	2,865 ±111	55,531 ±851	3,104 ±144	1,832 ±87

The response (±s.d.) of triplicate cultures of 25×10^3 A(B6A) 1-1 cells measured as c.p.m. of an overnight pulse with 2 μCi of ^{3}H-thymidine following 48 h co-culture with 1×10^6 irradiated (3,300 R) spleen cells (stimulator cells) of strains A/J(A), C57B16 (B6), (B6A)F_1, DBA/2 and SJL/J.

Studies by Cole and Nowell[5] suggested that repeated and prolonged proliferative activity would increase the probability of transformation in stimulated parental strain lymphocytes in a parent-into-F_1 G-v-H system. The use of alloreactive T-cell clones as the source of donor lymphocytes generates a unique situation in which the host is incapable of rejecting the donor cells, and the donor cells are in a situation of continuous stimulation and proliferation. As this clone apparently contains only Ly-1$^+$ 'helper' T cells (see below), induction of regulatory or cytotoxic T cells of donor origin would be unlikely and the donor cell proliferation would therefore be unregulated in B6A hosts.

The alloreactive T-cell clone A(B6A)1-1 selected for *in vivo* injection has been described previously[13]. Table 1 shows the reactivity pattern of clone A(B6A)1-1 on a small panel of stimulator cells. This clone has been carried for over 18 months *in vitro* without undergoing transformation and needs serial restimulation for propagation. At time 0, six separate mice, two each of strains A, B6 and B6A were injected intraperitoneally (i.p.) with 5×10^6 alloreactive T cells of this clone. One mouse from each of the three groups received sublethal (200 R) total body X-ray irradiation. Approximately 1 month after i.p. injection of clone A(B6A)1-1 cells, both hybrid B6A animals had large abdominal masses whereas neither the A nor the B6 animals showed any signs of tumour growth. Both B6A animals were killed and tumours, which seemed to be confined to the mesenteric lymph nodes, were excised and characterised as described below. The *in vivo* injected mice of strain A and B6 were killed 2 months after i.p. injection and none of the four mice showed evidence of tumour growth macroscopically, histologically or by attempted *in vitro* passage of lymphoid cells from their thymus, spleen or lymph nodes.

The lymphomas which arose in the two B6A mice were uniform in appearance. There was extensive replacement of lymph node elements by immunoblastic cellularity in mesenteric and pancreatic nodal tissues, and the spleen and pancreas were massively infiltrated by similar lymphoid cellularity (Fig. 1*a*, *b*). The immunohistological appearance of these lymphomas at the light and electron microscopic level will be reported elsewhere. The involved tissue was excised and the cells were passaged both *in vivo* and *in vitro*. Serological characterisation of the lymphomas for selected cell-surface determinants was carried out in the laboratories of Chella David at Mayo Clinic and Bonnie Matheson at NIH, by conventional techniques of complement-mediated cytoxicity, indirect immunofluoresence and absorption. The lymphomas derived from both mice were serologically identical and all were of cell-surface phenotype H-2K^k, 1a^a, Ly-1.2 and Thy-1.2 positive. They were surface immunoglobulin, H-2K^b, H-2D^b, 1a^b and Ly-2 negative. Preliminary studies on the virological status of these lymphomas using indirect immunofluorescence have shown that they are surface gp70 positive and p30 negative.

Despite continuous growth *in vitro*, on three separate serial *in vivo* passages the lymphomas derived from the original i.p. injection of B6A mice with clone A(B6A)1-1 would only grow in B6A mice (with or without X-ray irradiation). Repeated efforts to passage these lines in animals of strain A and/or B6 (with or without irradiation) failed to yield any tumour growth. Surprisingly, the tumour passed and cloned *in vitro* grew as a continuously replicating cell line. Thus, we have a perplexing situation which may be explained by two possibilities: either antigen drive is requisite for *in vivo* growth but not necessary for *in vitro* proliferation, or growth in B6A mice and the failure of these lymphomas to grow in strain A or B6 mice could be attributed to host regulation rather than lack of appropriate antigenic signal. This system supports the concept of lymphomagenesis occurring in a situation of antigen-driven lymphoproliferation. The model lends itself to extensive exploration as to the origin of the transforming event and the search for alloantigen receptors and/or cloned products on such alloreactive T cells, and has perhaps provided us with a useful tool for future hybridisation studies of immunocompetent T cells.

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Common pathways of interferon and hormonal action

J. Edwin Blalock & James D. Stanton

Department of Microbiology, The University of Texas Medical Branch, Galveston, Texas 77550

The action of interferon[1] as well as polypeptide hormones[2] has been shown to be transmissible between cells, possibly through gap-junctional transfer of secondary messenger molecules. This and other similarities between interferon and polypeptide hormones[3] have led us to propose that there is a common cellular pathway of interferon and hormonal action. If correct, this hypothesis would predict that interferon should cause a species-specific hormonal response and a hormone should induce tissue-specific antiviral activity. If these two responses are mediated by similar secondary messengers, they should be transmissible and cross-activate cells. Here, we show that interferon caused a species-specific hormonal response (noradrenaline-like stimulation of the beat frequency of cultured mouse myocardial cells). Noradrenaline induced an interferon-like antiviral state in mouse myocardial cells but not human amnion (WISH) cells. In conditions which demonstrate interferon-induced transfer of viral resistance, exposure of co-cultures of mouse myocardial cells and WISH cells to either human interferon or noradrenaline caused an increased beat frequency in the myocardial cells and development of antiviral activity in WISH cells, respectively. These studies strongly suggest common pathways of interferon and hormonal stimulation that are transmissible between cells.

Figure 1*a* shows the dose response of the spontaneous beat frequency of cultured mouse myocardial cells to noradrenaline and is in agreement with previously published results[2,4,5]. Increases in beat frequency also resulted from treatment of the cultures with mouse but not human interferon (Fig. 1*a*). The interferon concentrations required to cause a change in beat frequency are equivalent to those which induce antiviral activity and are well within the concentrations produced *in vivo*. The similarity of the slopes of the noradrenaline and interferon dose responses suggests that they increase the beat frequency by a similar mechanism. These results show that interferon can elicit a hormonal response in a species-specific manner.

Figure 1*b* shows that overnight treatment with noradrenaline caused a dose-dependent development of antiviral activity in myocardial cells. Interestingly, the maximal antiviral activity was observed at the same concentration (10^{-6} M) of noradrenaline as the maximal stimulation of beat frequency. This finding suggests a relationship between noradrenaline-induced increases in beat frequency and the development of antiviral activity. The noradrenaline-induced antiviral state was not as strong as that induced by interferon (0.7 to 1.0 $\log_{10}$ reduction in virus yield compared with 2.0 $\log_{10}$ reduction). As will be shown below, in identical conditions, noradrenaline did not cause antiviral activity in human amnion (WISH) cells, thereby demonstrating its tissue specificity. These findings demonstrate that in addition to its classical effect on beat frequency, noradrenaline treatment of its target (myocardial) cells causes the development of an antiviral state.

Table 1 shows that purified fibroblast interferon ($10^{8.5}$ units per mg protein) caused an increase in beat frequency. Additionally, the stimulatory effect of a crude interferon preparation was neutralised by specific anti-interferon antisera. In other studies, interferon preparations of varying purity increased the

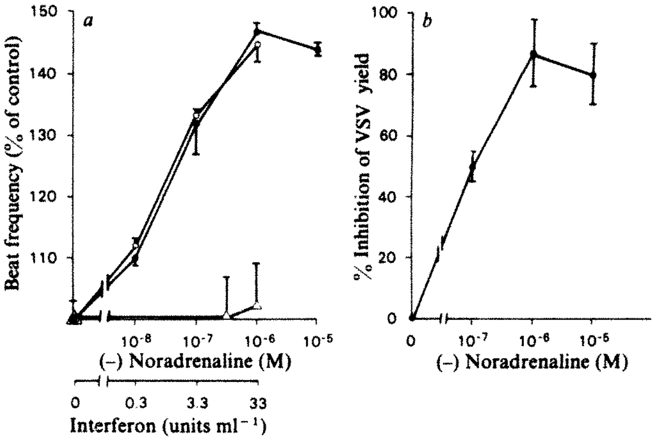

Fig. 1 Effect of (−)noradrenaline and interferon on the beat frequency (*a*) and virus replication (*b*) in cultured mouse myocardial cells. Except for the omission of filtering through nylon mesh, cells were prepared as previously described[16]. For the frequency experiments about 2.5×10^5 cells were plated into each well of Falcon multiwell tissue culture plates in 1 ml of Eagle's minimal essential medium (MEM) supplemented with 10% fetal calf serum, penicillin (100 U $\mathrm{ml^{-1}}$) and streptomycin (100 μg $\mathrm{ml^{-1}}$). After 24 h cultures were placed at room temperature for 1 h to equilibrate and experiments were carried out at this temperature. During the experiments the medium remained near *p*H 7.4. Each value is the mean ± s.d. ($n = 4$ cells in parallel culture). Beat frequency was determined visually with the aid of a microscope (×100) and stopwatch (Scientific Products). Each cell served as its own control. Control basal counts were 60–80 beats per min. Beat frequencies were determined 5 min after the addition of 0.1 ml of the indicated concentrations of each compound at room temperature. For virus replication studies 2.5×10^4 cells were plated into each well of Falcon microtitre tissue culture plates in 0.1 ml of culture medium. After 24 h medium was replaced with the indicated concentrations of noradrenaline in 0.1 ml volume in duplicate cultures and incubated overnight at 37°C in 4% CO_2 atmosphere. Supernatant fluids were removed and cultures were infected with vesicular stomatitis virus (300 plaque forming units (PFU) per culture). Virus yields were determined 24 h later by a previously described microplaque assay[1]. Control virus yields were about 1×10^6 PFU $\mathrm{ml^{-1}}$. Each point represents the mean % inhibition of the control virus yield ± s.d. ($n = 4$). ●, (−)Noradrenaline; ○, mouse interferon; △, human interferon.

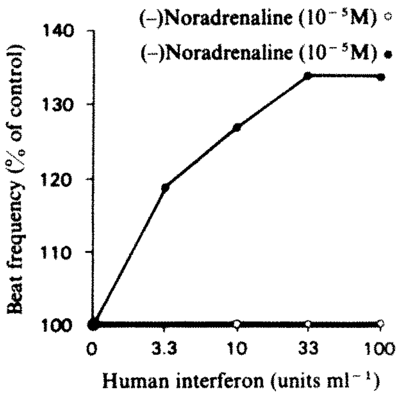

Fig. 2 Effect of human interferon on the beat frequency of mouse myocardial cells co-cultured with human amnion (WISH) cells. Mouse myocardial cells (2.5×10^5 per well) were plated into adjacent wells of a Falcon multiwell plate. Half of the wells then received 2.5×10^5 WISH cells. After overnight incubation, cultures were equilibrated to room temperature, human fibroblast interferon (1.8×10^6 units per mg protein) was added to achieve the indicated concentrations and the beat frequency ($n = 3$) determined as described in Fig. 1 legend. After beat frequencies had been determined, (–)noradrenaline (10^{-5} M) was added to cultures which had been previously treated with human interferon (100 units ml^{-1}) and the beats were again counted. ●, Myocardial cells + WISH cells; ○, myocardial cells.

beat frequency in direct proportion to their antiviral titre (data not shown). We also found (Table 1) that once myocardial cells were maximally stimulated with mouse interferon, addition of noradrenaline (10^{-5} M) caused no further significant increase. Also, interferon and noradrenaline increased the beat frequency with similar kinetics. Maximal stimulation with noradrenaline required about 1 min whereas interferon required 2 min (Table 1). This represents the most rapid reported final action of interferon. Taken together, these results strongly suggest that the effects of noradrenaline and interferon on the beat frequency of mouse myocardial cells occur through a common mechanism.

When the antiviral activity of noradrenaline was characterised, we found that the continued presence of noradrenaline and interferon was not required, as the antiviral state remained after overnight treatment and washing the cells to remove these substances. This shows that both interferon and noradrenaline act on the cell rather than on the virus. The interferon-induced antiviral state is inhibited by actinomycin D (refs 6, 7). Similarly, the induction of antiviral activity by noradrenaline was blocked by actinomycin D (Table 1). Like interferon, noradrenaline did not show virus-specific antiviral activity (see below and Fig. 3). In conditions of high multiplicity of infection, vaccinia virus is not sensitive to interferon[1] and was not inhibited by noradrenaline (data not shown).

The indication that interferon and noradrenaline share common pathways that may involve similar secondary messenger molecules implies that the hormonal activity of interferon and the antiviral activity of noradrenaline should be transmissible between cells. This hypothesis was tested with co-cultures of mouse myocardial cells and human amnion WISH cells. Human interferon did not increase the beat frequency of mouse myocardial cells (Figs 1*a*, 2). However, when human interferon was added to co-cultures of mouse myocardial cells and human WISH cells (which are sensitive to human interferon), there was a dose-dependent increase in the beat frequency of the myocardial cells. This effect was only observed when the myocardial cells were in contact with human cells. When noradrenaline (10^{-5} M) was added to co-cultures which had been maximally stimulated with human interferon there was no further increase in beat frequency, whereas noradrenaline addition to mouse myocardial cells treated with human interferon increased their beat frequency to that of the co-cultures (Fig. 2). The dose dependence of this response resembled that observed with mouse interferon on mouse myocardial cells (compare Figs 1*a* and 2). Both cell types in the co-cultures in the presence of human interferon also developed antiviral resistance whereas human interferon-treated mouse myocardial cells alone did not. Ten units of human interferon caused a 90% reduction in vesicular stomatitis virus (VSV) yield from mouse myocardial cells co-cultured with human WISH cells. Kinetic studies showed that the increased beat frequency due to mouse interferon treatment of mouse myocardial cells precedes by 1–2 min that observed in co-cultures treated with human interferon (data not shown). The observed sequence is consistent with the initiation of events in the human cell by human interferon, and their subsequent transfer to the myocardial cell. These data show that in conditions which can lead to transfer of interferon-induced viral resistance, transfer of the hormonal activity of interferon occurs.

The possible transmissible nature of the antiviral activity of noradrenaline was explored by infection of co-cultures with poliovirus, which only infects human cells[8]. Figure 3 shows that noradrenaline did not induce antiviral activity in human WISH cells alone. However, it produced a dose-dependent decrease in poliovirus yield from the WISH cells in the co-cultures. Poliovirus did not replicate in mouse myocardial cells in the presence or absence of noradrenaline, and co-culturing myocardial cells and WISH cells did not affect the yield of poliovirus from the WISH cells. Similarly, mouse interferon (50 units ml^{-1}) treatment of co-cultures (1 : 1 ratio) of mouse myocardial cells and WISH cells resulted in a 85% reduction in poliovirus yield from the WISH cells (data not shown). When VSV was substituted for poliovirus, the yield of virus from either cell type alone or in combination was similar. Noradrenaline caused a dose-dependent decrease in VSV yield from myocardial cells alone (Fig. 1*b*), but had no effect on the virus yield from WISH cells alone (Fig. 3). As the cells were mixed in a 1 : 1 ratio, if there had been no development of viral resistance in the co-cultured WISH cells one would have expected about 50% of the yield of a co-culture without noradrenaline. Figure 3 shows that noradrenaline caused a dose-dependent decrease in the expected VSV yield, indicating antiviral activity in the co-cultured WISH cells. Thus, noradrenaline causes the transfer of nonspecific virus resistance between its target cell and other cells.

Table 1 Characteristics of the hormonal activity of interferon and the antiviral activity of (–)noradrenaline on mouse myocardial cells

Addition to mouse myocardial cells	Beat frequency (% of control)*	Time required for maximum change in beat frequency (min)†	% Inhibition of VSV yield‡
Purified interferon§ (50 units ml^{-1})	142 ± 8	2	ND
Purified interferon (50 units ml^{-1}) + (–)noradrenaline (10^{-5}M)	161 ± 14	ND	ND
Crude interferon (50 units ml^{-1})	151 ± 3	2	99 ± 1
Crude interferon (50 units ml^{-1}) + anti-interferon antisera (1 : 20 dilutions)	88 ± 18	>10‖	ND
(–)Noradrenaline (10^{-5} M)	ND	1	75 ± 3
(–)Noradrenaline (10^{-5} M) + actinomycin D (2 μg ml^{-1})	ND	ND	0 ± 10

Procedures were as described in Fig. 1 legend. Crude interferon and anti-interferon antisera were mixed and incubated at room temperature for 30 min before addition to mouse myocardial cells. Beat frequency was counted. ND, not done.

* Values are means ± s.d.

† Times were derived from kinetics experiments as described in Fig. 3 legend.

‡ Procedures were as described in Fig. 1 legend. Noradrenaline and actinomycin D were added simultaneously. Values are means ± s.d.

§ $10^{8.5}$ Units per mg protein.

‖ There was no change in beat frequency after 10 min.

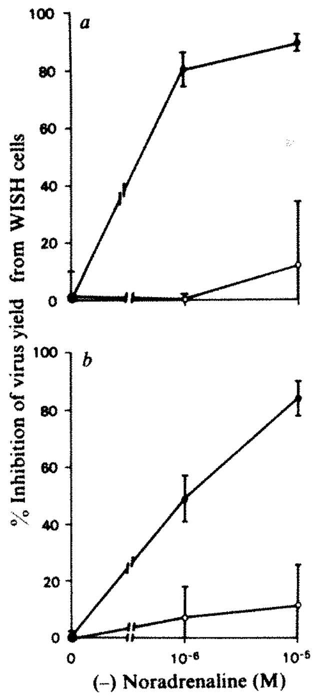

Fig. 3 Effect of (–)noradrenaline on virus replication in co-cultures of mouse myocardial cells and human amnion (WISH) cells. Mouse myocardial cells (3.0×10^4 per well) and human WISH cells (3.0×10^4 per well) were cultured alone or in combination (1:1 ratio) in Falcon microtitre tissue culture plates. After 24 h medium was replaced with the indicated concentrations of noradrenaline and incubated overnight. Supernatant fluids were removed and cultures were infected with 300 PFU of either VSV (*a*) or poliovirus (*b*). Virus yields were determined 24 h later by a microplaque assay[1]. Each point represents the mean % inhibition of the control virus yield ± s.d. ($n = 6$). VSV yield from myocardial cells alone was inhibited (Fig. 1*b*). Poliovirus did not replicate in mouse myocardial cells. ●, Myocardial cells + WISH cells; ○, WISH cells.

Thus, we have demonstrated that interferon can have hormonal activity and that hormonal stimulation can result in interferon-type antiviral activity. From these findings we conclude that interferon and hormonal action are probably mediated by common pathway(s). The transmission of the reciprocal actions of interferon and noradrenaline not only gives further credence to a common pathway of their actions but also suggests that common transferred molecule(s) are generated after interaction of either substance with the appropriate cell membrane. Superficially, cyclic AMP seems a candidate for the interferon-induced increase in beat frequency, because cyclic AMP can cause this response[2,9,10] and interferon in certain conditions can elevate cyclic AMP levels[11,12]. However, cyclic AMP alone cannot account for the antiviral effects as it is not antiviral[13] and interferon does not stimulate adenyl cyclase in all cells[11,12]. A more likely situation is that cyclic AMP and/or another small molecule(s) is responsible.

A question which results from these studies is, is interferon a hormone? The many similarities between interferon and polypeptide hormones indicate that interferon should be classified as such, and this is supported by our inability to distinguish interferon action from a hormonal response. As such, the natural role of interferon may be regulatory, with its effects on virus infections being secondary. The instances of low levels of interferon in normal individuals may not result from inapparent virus infections but may reflect this more general interferon regulatory mechanism. Additionally, this could be related to the side effects observed during clinical trials using high levels of interferon[14] as well as some aspects of viral pathogenesis. If interferon can cross-activate for other hormonal activities, this might explain the many diverse actions of interferon (for review see ref. 15). A shared mode of action of interferon and hormones would suggest that interferon action is not unique and makes questionable the view that the varied biochemical changes in interferon-treated cells are interferon specific.

A second question is, what are the limits of responses to hormones? The present findings suggest that hormones may, in addition to their known actions, protect tissues against viruses or maintain differentiation. If this could be documented *in vivo*, a new strategy of tissue-targeted antiviral and antitumour therapy might evolve.

We thank Drs J. Georgiades and S. Baron for helpful discussions. Purified mouse interferon and anti-mouse interferon antisera were supplied by Dr J. Georgiades and NIAID, respectively. This work was supported by US Army Medical Research and Development Command contract no. DAMD 17-78-C-8048.

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Corrigendum

In the letter 'Gravitational magnetism' by S.-P. Sirag, *Nature* **278**, 535 (1978), in Table 1 the maximum magnetic moment, *P*, for Her X – 1 should be 35×10^{26} A m^2.

Errata

In the letter by C. D. B. Bridges and S.-L. Fong, *Nature* **282**, 513 (1979), the title should have read: 'Different distribution of receptors for peanut and ricin agglutinins between inner and outer segments of rod cells'.

In the letter 'Oxidant damage mediates variant red cell resistance to malaria' by M. J. Friedman, *Nature* **280**, 245 (1979), the second sentence of Fig. 2 legend should read: 'The interaction of H_2O_2 and Hb results . . .'.

In the letter 'Inhibition of mixed lymphocyte response by monoclonal antibody specific for a rat T lymphocyte subset' by M. Webb *et al.*, *Nature* **282**, 841 (1979), the symbols in Fig. 1 have been wrongly given. The correct designation is: -●- w3/25 IgG, -○- W3/25 F(ab')$_2$, -□- W3/13 IgG.

In the News and Views article 'Is anyone out there?' *Nature* **281**, 528 (1979), two lines were inadvertently omitted leading to the attribution of views to I. Shklovsky entirely opposite to those he actually stated. The correct sentence should read: "I. Shklovsky of the Sternberg Astronomical Institute suggested that the choice of a value for *N is a function of the age of the investigator. He attributed 'youthful optimism' to T. Kuiper of the Jet Propulsion Laboratory who suggested that N may be a very large number* ($\gtrsim 10^9$).

MATTERS ARISING

Reevaluation of mitosis in the red alga *Porphyridium purpureum*

BRONCHART AND DEMOULIN have reported that mitosis in the red alga *Porphyridium purpureum* is 'unusual', with no kinetochores or well defined spindle pole bodies (SPBs), and they described a unique association of microbodies (MBs) with the spindle poles[1]. Our studies, however, lead to different conclusions and we report here some of our results.

P. purpureum, isolate 637 of the University of Texas Culture Collection of Algae, was grown in von Stosch enriched seawater medium under a 14 h light/10 h dark photo regime in 60-mm Petri dishes. Light microscopy showed 30% of the cells dividing 11 h after the start of the light period. Cells were fixed *in situ* at that time in culture media-phosphate-buffered 1% glutaraldehyde, postfixed in osmium, stained *en bloc* with 2% uranyl acetate, dehydrated in acetone, embedded in Epon, sectioned and poststained with lead.

Electron microscopy of serial sections showed that most dividing cells were in prophase. Each cell contained a large, stellate chloroplast and an eccentric nucleus. In early prophase a pair of SPBs was associated with the nuclear envelope (NE) in a region destined to become one of the division poles. Each SPB (Fig. 1*a*, *b*) had a relatively broad, elliptical portion proximal to the NE. In some micrographs it appeared attached to the NE by fine struts. The smaller, layered, cylindrical distal portion appeared connected to the proximal portion by an amorphous substance. Several MBs and a small zone of exclusion surrounded each SPB. Microtubules (MTs) appeared to originate near the zones of exclusion and increased in number between SPBs as one SPB migrated to establish the other division pole.

By metaphase the proximal portion of each SPB disappeared, while the distal SPB portion remained at the fenestrated poles and became closely associated with the intranuclear spindle MTs (Fig. 1*c*). Most MTs bypassed the chromatin, but several MTs were seen between the poles and chromatin, inserted on indistinct, but identifiable kinetochores (Fig. 1*d*).

During early anaphase the chromatin moved to the poles followed by a pronounced elongation of the interzonal spindle. The distal SPB portion and some extranuclear MTs were still present in early interphase.

It is understandable that kinetochores and SPBs were concluded to be absent in *Porphyridium*, although both appear to be evident in the published micrographs[1]. It was initially difficult to locate them even though we examined serial sections of many dividing cells. Kinetochores, because of their size and inconspicuous structure, were rarely encountered; and while SPBs were frequently observed in prophase, they were easily overlooked in subsequent mitotic stages when only the distal portion remained.

It is not possible to assign functional attributes to the MBs associated with the nuclear poles. This feature, considered to be unique to *Porphyridium*[1], has been observed in unicellular green algae where it is believed that the MBs are simply taking advantage of the spindle to ensure equal allocation to each daughter cell (K. Stewart, personal communication). We support this idea concerning the polar distribution of MBs because they are not known to be implicated with spindle formation or function and because there are other spindle-associated organelles which are now thought to be nonfunctional in nuclear division and are instead taking advantage of a 'free ride'[2,3].

Based on our observations, we conclude that the word 'unusual' is inappropriate and misleading to describe mitosis in *Porphyridium*, and should be used in

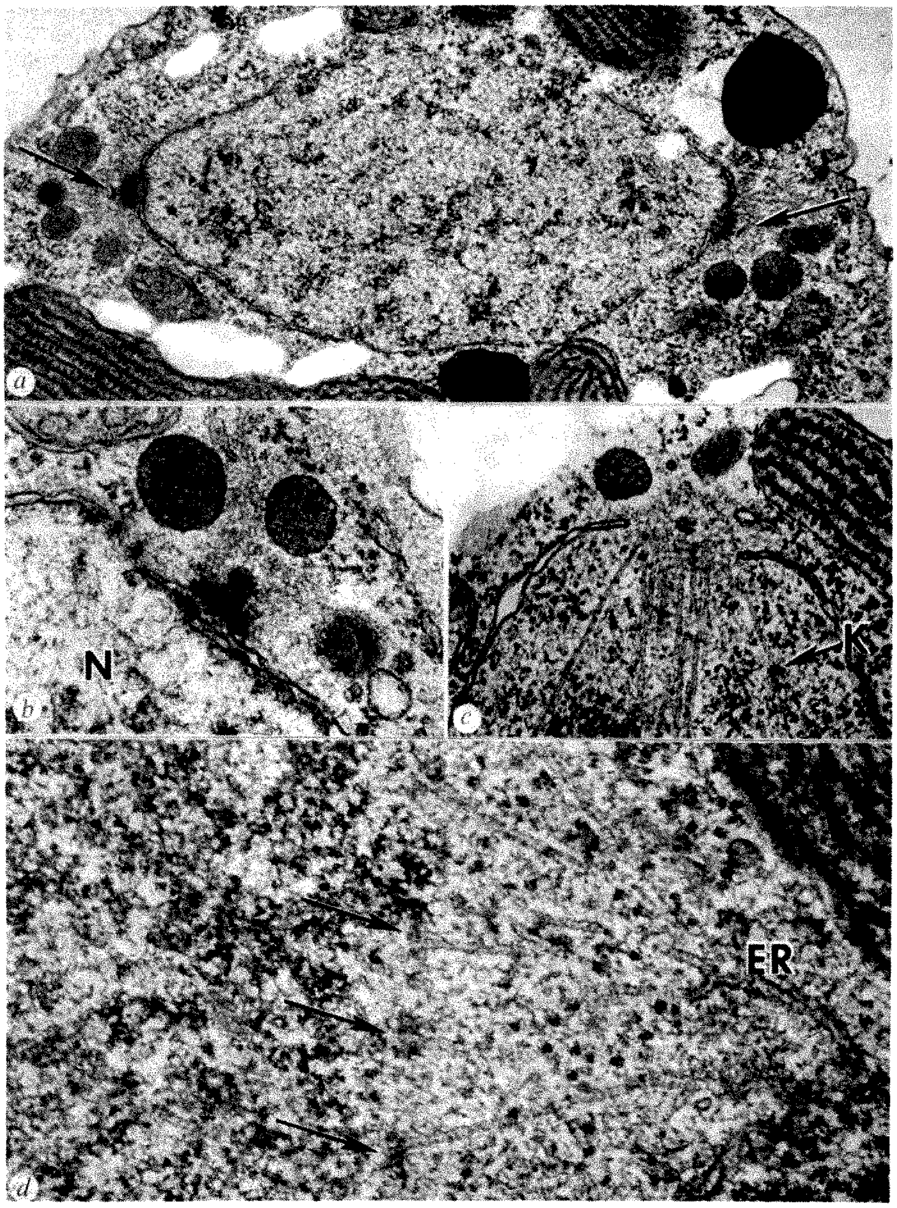

Fig. 1 *a*, Longitudinal view of late prophase nucleus showing established division poles. Each SPB (arrows) bridges a slight NE depression. The intranuclear spindle has not formed yet. The NE of this cell was apparently disrupted during specimen preparation, for it is well preserved in most cells (×33,750). *b*, Early prophase SPB. Note its association with the NE and MBs. Few MTs are present near the small zone of exclusion at this time. N, nucleus (×67,500). *c*, Distal portion of SPB at metaphase. K, kinetochore (×42,000). *d*, Tangential polar view of a metaphase nucleus showing kinetochores (arrows) with attached MTs. Endoplasmic reticulum (ER) is often seen at the poles (×90,000).

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characterising organisms whose mitotic mechanisms resemble those currently found only in primitive forms such as the hypermastigote flagellates, dinoflagellates and certain other protozoa[4]. Also, while there are minor modifications, the fundamental characteristics of mitosis in Bangiophycean and Florideophycean red algae appear to be the same (ref. 5 and our work in preparation with C. Bosco and J. Thomas). Furthermore, it has been suggested that an interrelationship exists between red algae and certain fungi[6,7]. The presence of morphologically similar, NE-associated SPBs in both groups, along with other common ultrastructural cell division features[4,8] may serve to strengthen this idea.

KATHLEEN L. SCHORNSTEIN
JOE SCOTT
Department of Biology,
College of William and Mary,
Williamsburg, Virginia 23185

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BRONCHART AND DEMOULIN REPLY—Schornstein and Scott have added some interesting observations to our data on mitosis in *Porphyridium*, and their interpretation of spindle pole body behaviour is especially welcome. Their main criticism of our paper seems to concern the use of the word 'unusual', a very subjective point. Our use of that word is due to the association of microbodies with the spindle poles, a fact confirmed by Schornstein and Scott. Neither unpublished reports of such a situation in some other algae nor speculation about its meaning change our opinion that our use of that adjective was legitimate. Our use of the word unusual did not result from a belief that either kinetochores or spindle pole bodies were absent.

Kubai[1] gives a lengthy table (Table 6) of organisms displaying microtubules terminating in chromatin without differentiation of kinetochores. We are aware that improved fixation and staining, or better luck in obtaining the right section, may eventually have revealed in those organisms, structures that might be described as kinetochores. Thus we did not stress the fact that we had not seen them in *Porphyridium*.

There is nothing unusual in the absence of a spindle pole body which is the rule among higher plants, but, as we said in our report, we observed and photographed (Fig. 2) such an organelle in *Porphyridium*. We spoke, however, of a microtubule-organising centre, for we were sure only of its presence in prophase and were not certain of its existence at the spindle poles in metaphase. We did not feel the small dark masses present at the poles in metaphase (our Fig. 1 and Fig. 1*c* of Schornstein and Scott) could qualify as a 'well defined pole body'. It is to the merit of Schornstein and Scott to have shown their constancy and relation to part of the prophase organelles. Those observations however do not make mitosis in *Porphyridium* closer to that in *Membranoptera*[2], because except for a qualification of the kinetochore situation (kinetochores are well defined in *Membranoptera* and inconspicuous in *Porphyridium*) every difference we noted remains true (presence of microbodies; lack of polar ring, well condensed chromatin and nuclear envelope doubling by endoplasmic reticulum). As to the relationship to fungi, there is certainly some similarity to the situation in Basidiomycetes. We already had that feeling in 1977 but did not stress it for fear of being accused of stretching the evidence in favour of a red algal ancestry for higher fungi, to which one of us is devoted.

R. BRONCHART
V. DEMOULIN
Département de Botanique,
Université de Liège,
Sart Tilman,
B-4000 Liège, Belgium

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Cancers and the immune system

EXCEPTIONALLY high levels of antibodies against the Epstein–Barr virus have been found in the blood of children who later developed African lymphomas[1], and this finding is widely regarded as evidence of a viral origin for these unusual tumours[2]. However, the tumours are little more than exceptionally localised (and easily cured) lymphatic neoplasms, and they are all but confined to areas of holoendemic malaria where the diffuse disease (lymphatic leukaemia) is conspicuous by its absence. In these same regions, over 50% of childhood cases of myeloid leukaemia have localised collections of myeloid cells (chloromas[3]) as the presenting symptom. Therefore, we could be witnessing the effects of general constraints on cancers of lymphatic and haematopoetic tissues in regions where a combination of high infection risks and low standards of nutrition makes the acquisition of exceptionally high levels of immunological competence during infancy a condition of further survival.

This hypothesis assumes that although the immune system may have more difficulty in recognising quasi-foreign cells (for example, mutants) than wholly foreign cells (microorganisms), nevertheless, it is playing essentially the same role in infective and neoplastic diseases. Therefore, an appropriate test would be one which compared the illnesses and immune reactions of European or American children with lymphatic leukaemia and more localised lymphatic neoplasms such as lymphosarcoma and Hodgkin's disease.

A. M. SETWART
G. W. KNEALE
Regional Cancer Registry,
Queen Elizabeth Medical Centre,
Birmingham, UK

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DE THÉ AND GESER REPLY—The comments of Stewart and Kneale on the contrast between the preponderance of localised lymphomas in tropical climates versus diffuse lymphoid malignancies in temperate climates are interesting. The prospective study carried out from 1972 to 1978 in Uganda showed that up to 4 years before Burkitt's tumour development, the future cases were marked by high antibody titres against Epstein–Barr virus (EBV)-specified viral capsid antigens, when compared with the general child population of the same age, sex and locality. The study further showed that other herpes virus antibody levels were not elevated in the Burkitt's lymphoma (BL) candidates and we consider that this specific stimulation of EBV antibody production contradicts the view implied by Stewart and Kneale that BL is caused by an exceptionally high level of immunological competence during infancy, rather than by a specific EBV-related event. As discussed elsewhere[1], Burkitt's lymphoma develops, as do most cancers, as the result of a multi-stage process, each step having specific causes. That high levels of immunological stress could promote the development of localised lymphatic neoplasms (BL) rather than leukaemias as seen in temperate climates, is an old and conceivable hypothesis, which does not contradict the involvement of a specific initiating viral event, as proposed for BL.

We agree with Stewart and Kneale that a comparative epidemiological, clinical and immunological study of lymphatic leukaemias versus localised lymphosarcomas, in temperate and tropical regions, would answer the question of whether children with localised lymphomas are more immunologically competent than those with diffuse lymphoid malignancies.

GUY DE-TÉ
CNRS, Faculté de Médecine,
Alexis Carrl, Lyon
and CNRS, Cancer Institute,
Villejuif, France

ANTON GESER
International Agency for
Research on Cancer, Lyon,
France

1. de-Thé, G. *Am. J. Epidemiol.* **1**, 32 (1979).

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REVIEWS

Impact of microelectronics

Andrew Holmes-Siedle

The Future with Microelectronics. By Iann Barron and Ray Curnow. Pp. 243. (Frances Pinter: London, 1979.) £7.95.

THE subtitle of this book, *Forecasting the Effects of Information Technology,* though less eye-catching than the main title, gives a better impression of its content. Here, in fairly raw form, is a report to Her Majesty's Government's Department of Industry. It contains the "intelligent extrapolations" of the two authors concerning probable impact of microelectronics over the next fifteen years. The Department certainly got what it needed. The authors have grasped the nettle firmly and given some serious criticisms combined with some quite radical recommendations for change in policy. To put their criticisms in a nutshell, the authors say that, so far, there has been talk only about the impact of microelectronics and that any practical moves made by the Government have all been small and poorly directed. As a result Britain, which has the capability of being in the top three is, in fact, seventh amongst the countries of the world in terms of progress in the use of microelectronics. The report provides a substantial number of the tools necessary to guide the Government in correcting this state of affairs.

In the 'Information Revolution' mankind has been granted a boon that it does not yet quite recognise. Swamped with information, man can now handle it at a low cost and with such ease that his life may greatly increase in quality. Unfortunately many see silicon microprocessors and memories as a threat rather than a boon. The revolution will impinge on business, on the factory floor, and on the home. Large sections of certain industries (for example, those making meters and electromechanical devices) may become redundant and this, if handled wrongly, could obviously be traumatic. Later on, even paper could be largely replaced by silicon. The benefits will lie in increased prosperity; a reduction of the anxiety felt today as we are presented with more information than we can handle; a greater opportunity for people to use their intelligence rather than their brawn; and better use of increasingly scarce material resources. The book under review does not spend time delineating these benefits. Its object is to describe how to reap them. Not only does it provide the forecasts demanded but, even more usefully, it details how Britain can improve its position in the field, using well reasoned argument.

One example of a tight technical argument is that provided on a strategy of production of silicon devices. The authors suggest that, within a short time, a generally adequate level of performance in integrated circuits will have been reached. When that point has been reached, British manufacturers should not continue trying to surpass their competitors in device performance but should instead attempt to extend their markets by a reduction in the price of the same devices. The authors note that this can best be done by reducing the number of rejects and devising a less labour-intensive way of encapsulating the silicon chips. By these means, a British firm could prosper against foreign competition. Such ideas might seem to represent nothing but conventional manufacturing commonsense but, for an industry so wedded to development of the silicon part of their product, the ideas have a revolutionary ring. Such arguments make it clear that at least one of the authors has considerable experience in the business of making microelectronic devices. Civil servants will be advised to listen well to such people before deciding policy. Makers of policy will also be helped by the effort, comprehensively made here, to carry their technical arguments logically into the sphere of industrial aid policy. Perhaps one of the most important items in the report is a recommendation that the government shift their financial support away from research and towards assisting the actual sales of devices; moreover, the authors recommend that support be shifted away from Government research establishments and towards industry laboratories. I would imagine that the aim of this recommendation is not primarily to put an axe into research but rather to increase substantially the whole level of Government funding of microelectronics, keeping the research level constant but helping to promote specific ranges of the equipment which has been improved by the use of microelectronics. Such a direction could, the authors hold, much more surely increase Britain's share of the market in several highly profitable fields, such as office equipment (the authors see clerical work as one of the fields most prone to be changed by microelectronics in the near term). Again, the idea might seem obvious did not the authors note that many Government committees find it more congenial to fund research than to support the manufacture of end-products. Though stated kindly and factually, the implication of the authors is clear: some of our civil servants have, in this field, tended to steer clear of hard decisions which involve the fate of a business. I would welcome a longer book which includes some discussion as to why this should be so, and how British practice compares with that in, say, France and the USA. Is it a lack of technical appreciation of the microelectronics industry, aggravated, perhaps, by the great speed with which it is developing? Or is it a fundamental characteristic of civil servants to avoid the more critical gambles? If so, will a report, like this, however convincing, conquer that tendency?

For the general reader, the main problem with this book will come with the style, which is that of a technical report. In the interests of bringing the report quickly to a wider public, it would seem that little time was spared for making it more intelligible to that public. The one superficial addition, a glossary of technical terms, is a particularly weak effort. In fact, many definitions miss the point and some of the more obscure terms in the text are not covered. Moreover, the authors sometimes favour Americanisms (what is an "implementation house"?) and adjectival phrases ("handling, machining, forming and labour intensive"). It is a pity that an important statement of the future, as I believe this is, may only further baffle the general public.

Does the book give a well-rounded picture of the next fifteen years or more? Here, it may be said that the authors' immersion in one technology may work against them. They are clearly "silicon men" and claim that silicon devices of one class, n-channel MOS devices fabricated from single-crystal silicon, can answer all of our needs in microelectronics, at least in the medium term. They therefore give virtually no time to considering the potential technical advances in other branches of semiconductor technology, such as that of gallium arsenide and certain thin-film structures. Even in the medium term, such devices could possibly both outperform *and* underprice silicon devices. I would most certainly agree that there is ample room for the expansion of the use of silicon devices in low-speed microelectronics (for example, a silicon diary could inform one of tomorrow's appointment and still operate at a very low data rate); but other growing fields, such as mass data-processing, demand such high switching speeds that the designers of fast computers already claim that silicon is "running out of capabilities". This, however, is one of the few points which is insufficiently argued in a book which, without doubt, is an important contribution to the debate on Britain's role in the Information Revolution. The highly condensed statement whcih they give leaves room for several further books which expand on the issues so succinctly raised and it is to be hoped that this statement will be a first step in a wider, more sophisticated, debate on the impact of microelectronics on our future lives. □

Andrew Holmes-Siedle is Consultant to the Physics Department of the Fullmer Research Institute in the fields of solid-state physics and radiation physics.

Ageing research

I. Davies

The Biology of Senescence. Third edition. By A. Comfort. Pp.414. (Elsevier: New York, 1979.) $19.95.

THE study of ageing is now taking place on a large scale throughout the world. There is a rapid proliferation of new books with "overviews" of the subject to date. The second edition of Dr Comfort's book was produced in 1964 and at that time was one of the few texts analysing senescence in a variety of animal species, and also presenting a critical survey of current theory in the field of ageing research. One rather expected that the latest edition of this book would attempt to carry out the same task, but with an updated review of the current literature. However, the end product is rather a strange hybrid, a very thorough analysis of early research in the ageing field, followed by a somewhat scanty examination of the later literature.

Much of the text is easily recognised as being from the earlier edition and contains an interesting description of the historical and philosophical background of the study of the ageing process. There is a mass of data on the maximum life-spans achieved by animals from most of the major phyla, together with discussions of the various measurements (morphological, biochemical, physiological, and so on) made on such ageing populations. The influence of genetic constitution and growth on senescence is also described. Much of this information is valuable and informative, but the majority of the references cited are pre-1964 with hardly any reference to the wealth of recent data.

Various other defects are also apparent — such as non-matching of references in the text with those in the extensive bibliography, and also examples of changes in symbols between diagram and legend — but presumably these can be blamed on the publishers.

The remainder of the book examines the proposed mechanisms of senescence, a discussion of the position of ageing studies, and a call for the direct measurement of human ageing. This is the most recent section of the text and contains a very brief review of current concepts in the field of gerontology. The discussion is too short and is not critical enough. In addition, there is some rather blatant grafting into the previous edition with a close juxtaposition of statements such as "antibodies are now thought to be produced by lymphocytes" and then more or less detailed descriptions of current research on immunological factors in the ageing process. The scantiness, and the choice, of the references used to question (for example) the blind acceptance of senile neurone loss, is also doubtful. Similar criticism could be levelled at other topics.

Overall, I found this book very disappointing. There is a good deal of useful information which would be of use in establishing background for graduate course work in a study of senescence. However, because of the less than satisfactory updating of the book it would be of limited use for a review of current research into the ageing process. □

I. Davies is a Research Associate of the Geigy Unit for Research in Ageing at the University Hospital of South Manchester, UK.

Basic physics of X-ray spectroscopy

D.S. Urch

X-Ray Spectroscopy: An Introduction. By B.K. Agarwal. Pp.418. (Springer: Berlin, Heidelberg and New York, 1979.) DM74; $40.70.

X-RAY SPECTROSCOPY is enjoying a renaissance with the development of techniques such as extended fine structure (EXAFS), with the availability of high-resolution spectrometers and with the construction of powerful sources of synchrotron radiation. It is therefore a pleasure to welcome Professor Agarwal's new introduction to the subject. Of necessity much of the book is concerned with the basic physics of X-rays and this involves the presentation of a considerable amount of historical material. This is done in a clear and logical way with full mathematical derivations. Indeed, if there is a general criticism of this book, it is that there is possibly an over-emphasis on mathematical rigour at the expense of descriptive explanation.

The first three chapters are devoted to a description of X-electromagnetic radiation, to characteristic X-rays and to the interaction of X-rays with matter. This latter subject then leads on to specific topics of current interest. The value of the book is greatly enhanced at this stage by a consideration of the photoelectric and Auger effects, and the consequences which these processes have for X-ray spectroscopy. Peak widths and the origin of the non-diagram (satellite) lines are dealt with in detail. As high resolution X-ray spectroscopy in both emission and absorption can shed considerable light on the electronic structure of metals, alloys and other solid materials as well as molecules, this 'chemical' application is described in some depth. The various types of chemical bond are described and there is a brief introduction to molecular orbital theory so that chemical shifts of X-ray emission peaks can be rationalised. The fine structure near X-ray absorption edges is discussed in the same way and a basic account is given of the EXAFS technique and of the information that can be derived from it. Even so, it is unfortunate that in this chapter (as in others) very few recent references are to be found (less than 10% of the references are post-1970). This lack is particularly felt in the chapter on the fast growing area of soft X-ray spectroscopy where fundamental advances are either omitted (for example, Henke X-ray tube) or barely mentioned (for example, grazing incidence spectrometer).

General experimental methods in X-ray spectroscopy are dealt with quite briefly, only the basic physical principles being explained in detail. The emphasis in this book is theroretical, rather than experimental, but it has merit as an introduction to the basic physics of X-ray spectroscopy and to its applications. It should therefore form a valuable addition to the libraries of X-ray laboratories and could be used in postgraduate courses in X-ray physics. □

D.S. Urch is Reader In Physical Chemistry at Queen Mary College, University of London, UK.

Soluble cell products in the immune response

Anne S. Hamblin

Biology of the Lymphokines. Edited by S. Cohen, E. Pick and J.J. Oppenheim. p.626. (Academic: New York, San Francisco and London, 1979.) $42.50; £27.60.

THE word lymphokine was originally invented to denote those cell-free soluble factors, other than immunoglobulins, generated *in vitro* during the interaction of sensitised lymphocytes with specific antigen, and with biological activities consistant with them functioning as regulators of immune responses *in vivo.* Subsequent work has shown that the same, or similar, activities may be produced from other cells of both lymphoid and non-lymphoid origin under a variety of circumstances. The editors have therefore taken the view (with which there might be some disagreement) that their terms of reference for topics discussed in this book should be wide, and include "soluble cell products of all categories to which a function in the central or peripheral regulation of the immune response has been attributed, with the exception of classical antibodies". Within the potentially enormous list of substances which could be discussed in this context, the editors have made a clearly stated selection of those which will receive attention and those which will not. This book is therefore about the activities of some soluble cell products, mostly derived and demonstrable *in vitro,* which are at present considered relevant to the regulation of immune responses *in vivo.* However, it is not simply a list of factors, but more interestingly an attempt to focus on some aspects of current lymphokine research, and in particular mechanisms of action.

The book consists of twenty chapters by different authors, most of whom have considerable experience of experimental work with lymphokines. The first, by the editors, entitled "The lymphokine concept", clearly explains the problems surrounding the definition, origin and relevance of lymphokines, and sets the stage for the ensuing chapters. The second entitled "Lymphokines as inflammatory mediators", includes a useful discussion of all the available, compelling, although inconclusive, evidence that these substances, the activities of which are based on *in vitro* assays, do have a role *in vivo,* and provides a basis for argument with those who regard lymphokines as test-tube artificats. Thereafter follow sixteen chapters, some of which review 'classical' lymphokine activities (migration inhibitory, cytotoxic and mitogenic factors) and some of which describe more recent fashionable additions to the lymphokine repertoire (factors affecting T cell - B cell cooperation, antigen-specific regulatory factors, and specific and non-specific suppressor T cell factors). In addition, there is an interesting chapter by L. Epstein comparing the properties of classical and immune interferons, a chapter on the important and difficult problems of purification and characterisation of lymphokines, and several chapters on lymphokine activities derived from a variety of cell sources of both lymphoid and non-lymphoid origin. The book concludes with two general reviews; the first a stimulating article on the role of intracellular mediators in the immune response by C. Parker, which although it makes little reference to lymphokines, was presumably included on the basis that the editors feel that future research on their biology may focus on such mechanisms; and the second an overview of the biology of lymphokines by B. Waksman.

The topics selected for inclusion in this book reflect current aspects of the study of lymphokines which the editors view as important. Within our present state of limited knowledge about soluble cell products involved in immune regulation, and in particular their biochemical purification and characterisation, the relative importance of the subjects discussed remains a matter of speculation. Nonetheless, the book brings together information and concepts which can form the basis of useful discussion amongst those actively involved in lymphokine research, as well as those more generally interested in biological control mechanisms. □

Anne S. Hamblin is Lecturer in Immunology at St. Thomas' Hospital, London, UK.

Simple qualitative molecular orbital theory

Anthony Stone

Molecular Structure and Bonding: The Qualitative Molecular Orbital Approach. By B. M. Gimarc. Pp. 224. (Academic: New York, San Francisco and London, 1979.) £11.70.

THIS book endeavours to explain how a knowledge of simple qualitative molecular orbital theory can give a detailed understanding of the shapes of molecules. It is rather limited in scope, covering only H_3, H_4, AH_2 to AH_4, AB_2 to AB_6, HAB and H_2AB, A_2H_2, A_2H_4 and A_2H_6, and dimers of AB, AB_2 and AB_3 (where A and B are first or second row elements; there is no mention of transition-metal compounds) but these molecules are discussed in considerable detail, with many references to experimental and *ab initio* data. The book is aimed at senior undergraduates or first-year graduates, though it is not entirely consistent in level: for example, it is not thought necessary to explain what is meant by the Hartree-Fock method with configuration interaction, but the simple molecular-orbital picture of H_2 is discussed in some detail. Similarly the reader is not expected to know or learn any group theory beyond "a knowledge of symmetry classifications", but the molecular orbitals of the various species are given, in qualitative form, without any hint of the group-theoretical procedures used in their derivation. Such theoretical explanation as is given (for example, of the change in the form of the orbitals on bending on AH_2 molecule) is woolly and unconvincing. Students who had not met Walsh diagrams before would have difficulty with the treatment of AH_2, let alone the more complicated examples.

However, the main limitation of this book is its single-minded commitment to one approach. Changes of energy on passing from one structure to another are to be viewed wholly in terms of changes in overlap. There is no mention, in the main text, of Walsh, whose classic series of papers dealt with most of these molecules 25 years ago, or of the Jahn-Teller effect or the pseudo-Jahn-Teller effect. The only concession to other methods of tackling these problems is a couple of pages at the end of the book, in which other approaches are briefly mentioned, almost as if to counter the accusation that the author has never heard of them, but there is no attempt whatever to integrate these ideas into the book as a whole. In the discussion of H_4 for example, three possible structures are considered, namely tetrahedral, square-planar and linear. The overlap criterion is used to rank these in order of energy, and it is concluded, not very convincingly, that the linear structure lies lowest. But application of the Jahn-Teller theorem shows conclusively that the ground state *cannot* be tetrahedral or square-planar, because either of these structures would, according to the theorem, distort spontaneously. The reader should be told about this far more rigorous approach.

In summary, the book contains a good deal of useful information on a rather restricted subject, but it is discussed from a very narrow point of view. A disappointing book. □

A. J. Stone is an Assistant Director of Research in Theoretical Chemistry at the University of Cambridge, UK.

Blood-brain barrier

Stanley I. Rapoport

The Concept of a Blood-Brain Barrier. By Michael Bradbury. Pp.465. (Wiley: Chichester, UK, and New York, 1979.) £22.

THIS book by Michael Bradbury, of King's College, London, is a scholarly, comprehensive and readable analysis of past and recent studies on the blood-brain barrier (BBB) by a researcher who has made major contributions to the field. It extends the important review by Davson (*Physiology of the Cerebrospinal Fluid*, Churchill, Livingstone: Edinburgh, 1967) and complements, with only slight overlap, my own book (*Blood-Brain Barrier in Physiology and Medicine*, Raven: New York, 1976). It contains more than 1,000 references, and should be of value to researchers as well as to neuropharmacologists, neurochemists, neurosurgeons, neurologists and transport physiologists.

The BBB separates the brain and cerebrospinal fluid (CSF), on the one hand, from blood on the other, and is a regulatory system consisting of three tissue sites — the cerebrovasculature, the choroid plexus and the arachnoid membrane. Each site contains at least one cell layer that is connected by tight junctions (which restrict intercellular diffusion of water-soluble drugs and proteins) and that has specific transport mechanisms that help to regulate the ionic and water contents of the brain, and to facilitate delivery to the brain of critical substrates for metabolism and synthetic processes.

Chapter 1 introduces the anatomical and functional relationships between barrier tissue sites and brain cells. Chapter 2 summarises early studies on the barrier to intravascular dyes and colloids, and presents methods of compartmental analysis that can be used to estimate cerebrovascular permeability. The vasculature is selectively permeable to lipid-soluble as compared to water-soluble agents. Chapter 3 reviews historical controversies about BBB sites, and shows that the BBB at the cerebral vasculature arises at the continuous layer of endothelial cells that have little pinocytosis and are connected by tight junctions. The chapter also analyses methods of measuring cerebrovascular permeability *in vivo*, and discusses capillary hydraulic and water permeability. Bradbury concludes that functional pores exist at the vascular endothelium for non-electrolyte diffusion, although I believe that evidence suggests that they do not. The sink action of CSF, in washing material from the brain extracellular space, is discussed.

Chapter 5 indicates that apparent regional differences in blood-brain exchange reflect differences in capillary surface area between white and grey matter. It considers the special, high permeability regions in the brain in detail (that is, median eminence, posterior pituitary), which have vessels with porous, fenestrated endothelia and have neuroendocrine and chemoreceptor functions. Blood vessels in the retina, optic nerve and peripheral nerve also have a continuous endothelial lining.

Chapter 6 elaborates the properties of saturable, stereospecific mechanisms at cerebral capillaries for facilitated diffusion of sugars, amino acids, ketone bodies and monocarboxylic acids. Bradbury suggests that amino acids are transported actively from brain to CSF, although the evidence for this is weak. Chapter 7 critically evaluates experiments designed to prove active transport at the cerebral capillary, and finds most of them wanting. The best one is that by Davson and Hollingsworth with ^{131}I.

Chapters 8 and 9 summarise much of Bradbury's original studies on homeostatic regulation of ions within the nervous system. The apparent selectivity of the cerebral capillary to K^+ as compared to Na^+ could be due to active K^+ transport or to exchange diffusion of K^+. Transport at the choroid plexus maintains CSF concentrations of Ca^{2+}, Mg^{2+}, Na^+ and K^+ within strict limits, despite marked changes in plasma concentrations. A sigmoid relationship between CSF K^+ and K^+ efflux at the plexus, and a K^+-independent influx, could stabilise CSF K^+. Brain and CSF *p*H are also stabilised through modification of choroidal secretions and brain organic ion content (especially in alkalosis). Control of brain ionic content helps to maintain a constant brain volume during a chronic change in plasma osmolality.

BBB phylogeny is discussed in Chapter 10. BBB structures are in place but are less tight in the immature than in the mature brain. Regulatory mechanisms for specific ions mature at different rates, but are completely developed during the final spurt of brain growth.

Chapter 11 discusses the BBB to drugs, neurotransmitters and metabolites. Most current methods are not sufficiently sensitive to measure capillary permeability of slowly permeating agents, such as peptides. Clinical depression and schizophrenia are related to CSF concentrations of specific neurotransmitters or their metabolites. CSF concentrations of folic acid, vitamin C, peptides and morphine are regulated by choroidal transport.

Chapter 12 summarises effects of different insults on BBB permeability — hypercapnia, seizure, hypertension, toxic agents, hypertonic solutions. Bradbury concludes that the possible mechanisms that might increase cerebrovascular permeability — tight junctional widening, increased vesicular transport, transendothelial channel formation — remain to be evaluated critically. The resistance of the BBB to hypoxia and ischaemia is emphasised, as are principles underlying cerebral oedema. Chapter 13 summarises overall BBB function. A final proof reading of the book would have avoided a few trivial errors. □

S.I. Rapoport is Chief of the Laboratory of Neurosciences at the National Institute on Aging, NIH, Baltimore, Maryland.

Birdwatching postscript

C.M. Perrins

The Mitchell-Beazley Birdwatchers' Pocket Guide. By Peter Hayman. Pp. 192. (Mitchell-Beazley/RSPB: London, 1979.) £3.95.

THIS is a slender, wallet-sized book which can be conveniently carried with one into the field. It is crammed full of coloured sketches, most of which are very small (less than an inch across), but they usefully portray the birds in a variety of plumages and poses (perched and in flight) and from different angles. Juvenile plumages are also often shown, though I would have preferred more of these to have been included.

The text is very sparing but includes the most important features of each species, their calls, their habitats, and range. Some of this information is given in the form of symbols, thereby saving space. There is also usually a small silhouette of each species against that of either a sparrow or a pigeon in order to give a quick idea of size. This is not a total success; when the bird in question is very large compared with scale model (for example, pheasant with sparrow or eagle with pigeon) the model may be less than 2 mm in size. In such cases, the poor reader is left to determine which of these two models this tiny spot of ink is supposed to represent!

The book covers some 350 species and hence includes almost all the birds of Western Europe. More detail is given for those which are commonest in Britain than for the rarer birds; the latter are sometimes grouped together on a page and so are not located with their closest relatives. The order used is not an accepted taxonomic one, but one designed to help the beginner locate the species concerned. On the whole this is a well produced, highly portable little book which many will find very useful. □

C.M. Perrins is Director of the Edward Grey Institute of Field Ornithology, University of Oxford, UK.

nature

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Scientific exchanges must serve science, not the Soviet government

THE exile of Dr Andrei Sakharov, coming so soon after the Soviet invasion of Afghanistan, raises once again the problem of a scientist's duty to take a stance on world affairs. Ironically it comes this time in defence of a man whose actions, over the past twelve years, have made it inevitable that scientists should be expected, by the public at large, to have such a stance. Just earlier this week, he issued a statement from internal exile in Gor'kii, via his wife, calling for a fair and democratic society and the defence of human rights. At the same time he accused the Soviet government of repression.

It would be simplistic, and far from correct, to attribute to Sakharov's influence the current concern of scientists with the social consequences of their research. Nevertheless, the appearance in *samizdat* in 1968, and subsequent publication in the West, of Sakharov's "Thoughts on Progress, Coexistence and Intellectual Freedom" have added a new dimension to the public image of the research scientist. Instead of what Isaac Asimov had called the "Frankenstein syndrome", the public were presented with a nuclear physicist, "father of the Russian H-bomb" (a title which Sakharov himself has always denied) who had turned over all his earnings from the weapons programme to cancer research, and begun a lone campaign for increased intellectual freedom and international exchange as a means of overcoming international tensions and confrontation.

In later years, Sakharov was to criticise this essay as immature. Certainly, as he became more closely identified with the growing wave of intellectual unrest in the Soviet Union, his approach to the problems of *détente* became more profound and, perhaps, less trusting of official statements of intent. The appearance of his essay in 1968, the "year of Czechoslovakia", however, threw a fundamentally new light on the Soviet scientific establishment. Hitherto, dissent had largely been confined to literary and student milieux, and Soviet scientists had been seen, at any rate from abroad, as enjoying an elitist position, with all the many privileges that the "classless" Soviet society gives to those it wishes to cherish.

For a Soviet scientist to risk his job, position and freedom, for the sake of an ideal has led, inevitably, to a new focus on the problem of the scientist's personal responsibility for the use to which his or her research is put — and, more generally, his or her obligations to fellow scientists and to humanity at large.

Dismissal from employment compatible with Sakharov's background and abilities came swiftly, providing the Soviet media with the chance of presenting him as a "drop-out" who has done no real science for the past 12 years. Even if this were true — and the circumstances of his arrest last week, on the way to a physics seminar, suggest otherwise — Sakharov's former scientific achievements and his continuing membership of the Academy surely constitute at least as much a claim for support from the scientific community as they would in the case of an aged and long retired professor emeritus. The storm of protest telegrams from scientific bodies and individual scientists indicate that this obligation has been felt and answered.

What form, apart from verbal protests, should this concern over Sakharov take? Already the US government has cut back on technology transfer to the Soviet Union — and this at a time when the new Five Year Plans are being completed — plans which would have originally assumed the availability of US knowhow and/or hardware. Although the Soviet government will admit only Olympic visitors this summer, there are a number of international conferences scheduled to take place in the Soviet Union, before and after the Olympic shut-down, as well as an impressive programme of exchange visits and joint projects. Most significant, next month, the Hamburg Scientific Forum meets to discuss the implementation, as regards science, of the Helsinki Accords.

Since the first plans were made for Hamburg, the irony of the case of Yurii Orlov, has been observed on a number of occasions. Orlov, a physicist and founder of the Moscow "Helsinki Monitoring Group" is in a Soviet labour camp precisely for attempting to report on breaches of these accords. A number of western human rights activists, notably Valentin Turchin, advocate boycott of Hamburg until such time that Orlov is free to attend. Sakharov's exile, they claim, makes the case for boycott even stronger. At the other extreme lies the "soft diplomacy" approach: keep the contacts going, and try to do what one can behind the scenes. A doctrine which can all too easily become an apologia for inaction.

It would be ironic if, in defence of Sakharov, the world scientific community denies itself vis-a-vis the Soviet Union, all forms of international exchange which Sakharov himself advocated. On the other hand, it would be idle to pretend that the reprisals against him make no difference.

If scientific exchanges are to continue, then they must be seen to serve science and scientists — not the propaganda interests of the Soviet government. This, surely, is the time to implement a policy of "accept no substitutes"; to turn away from conferences those uninvited participants who turn up, armed with an irrelevant and insignificant paper, in place of the invited speaker whose passport somehow failed to materialise; and to protest against the terms of the form, signed by every Soviet scientist leaving the country, which limits his discussions to materials already published or passed for publication in the "open journals", thus cutting out the discussion of work in progress which is one of the most valuable features of any scientific meeting. And, when attending conferences in the Soviet Union, to make every effort to contact one's opposite numbers who may have been excluded from the conference — even if taking place in their own speciality and in their own home town — as happened a few years ago to the Goldshtein brothers.

Finally, when the occasion arises in the course of such conferences and contacts, scientists should relentlessly press for the implementation of those human rights agreements for which Sakharov has become, *par excellence*, the symbol of the concerned scientist. Indeed, perhaps the greatest tribute to his work is that the Soviet authorities now expect scientists who go abroad for conferences or exchanges to be challenged on such matters. In signing the pledge form referred to above, the Soviet scientist officially commits himself to answering all such enquiries according to the principles laid down in the official Party press. ☐

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1981 budget reverses shift from physical sciences to life sciences

Defence research increases set the pace for a 13% growth in the R&D budget which President Carter is asking from Congress in 1981. **David Dickson** reports from Washington

THE Carter administration is seeking to use the 1981 budget to reverse what it sees as a "disturbing trend" in science policy over the past decade and a half, namely a relative decline in funding for research in mathematics and the physical sciences compared to the growth in medical research and life sciences.

Official calculators, for example, show that support for physics dropped by 13.5% in real terms between 1967 and 1980, and for mathematics by 16.7% over this period. In contrast funding for the life sciences increased by 19%, and the environmental sciences experienced a real growth of 33.7%.

Moves to correct this imbalance — for example within the budget of the National Science Foundation, which plans to give particular emphasis to increasing support for fields such as computer science — figure prominently in a budget request also characterised by a growth in research projects jointly sponsored by the federal government and private industry, and dominated by significant increases in Defense Department expenditure on research.

Overall President Carter, who singled out basic research in his budget message to Congress last Monday, as one of the few areas meriting increased support, is asking Congress for a 13% growth in support for research and development, including a 12% increase in funds for basic research.

Given an officially-estimated increase in cost of 9% (a figure some claim to be overly-optimistic) this will mean real growth in 1981 of 4% and 3% respectively in the total R&D and basic research expenditures.

Much of this is directly due to increases in the military research budget. Military sponsored R&D is planned to grow by 20.2% — including funds for research in very high speed integrated circuits and for full scale development of the MX intercontinental ballistic missile — and military support for basic research will be up by 21.4%.

The growth in the budget of the National Aeronautics and Space Administration will be comparatively smaller, at 9.8% for overall R&D (taking into account an extra $300 million being requested for 1980), and 11.5% for space science.

However Dr Robert Frosch, Administrator of NASA, calls it a "good start" to the decade, with growth in all areas of the budget, at least compared to last year when the extra costs of the space shuttle meant no new space science programme starts.

Included in NASA's request for 1981 are funds for a gamma ray observatory, and an operational National Oceanic Satellite System (NOSS), which will build on the success of last year's SEASAT mission, and will be jointly funded with the Department of Defense and the National

Energy department announces 'slip' in laser fusion

THE Department of Energy has decided to allow "slippage" in construction work on the NOVA neodymium glass laser and irradiation facility at the University of California's Lawrence Livermore Laboratory in order to ensure a "more balanced" programme of advanced driver studies for inertial confinement fusion technology.

Construction work on NOVA started last year, and over $80 million will have been spent on the project by the end of the current fiscal year. However in its budget request for 1981, the department has included no extra construction money — although it says that construction work will continue. Final costs are estimated at $136 million.

In contrast to inertial confinement, the budget for magnetic fusion research has been increased by 13.5%, to a total of $403.6 million. This figure includes a 40% increase for development and technology, and a 36% increase for applied plasma physics.

NSF to fund laboratory renovations

THE National Science Foundation is planning to introduce a new programme to help universities renovate out-dated research facilities. Federal funding for the first year of the programme will be $14.3 million, and the money will be awarded on a matching-grant basis, with academic institutions matching federal support with funds from non-federal sources.

According to NSF director Dr Richard Atkinson, the money will not be available to buy new instrumentation or equipment, or to restock libraries, but is intended to be spent on the improvement of existing facilities, on the basis that outdated facilities "bring less than optimal return on the investment of federal dollars for support of research". A statement from the Office of Science and Technology Policy says that the cost-sharing programme will focus on "the nation's most productive research universities".

Defense Department increases university research support

THE Department of Defense plans to increase by about 25% in 1981 its support of both research and development in general, and basic research in particular, carried out by US universities.

Figures released by the Office of Management and Budget indicate that DOD support for university research is expected to increase only 10.5%, from $401 million to $443 million, part of an overall 9.5% increase in federally-funded university research predicted to reach a total of $4.6 billion.

However Dr Frank Press told a press briefing that according to Dr William Perry, Defense Under-Secretary for Research and Technology, new programmes planned by the DOD will mean that the actual increase is more than twice this rate. Overall support for basic research by the Defense Department is proposed to increase by 21% in 1981.

Energy research: less for nuclear, more for coal

PRESIDENT Carter is recommending to Congress that the Department of Energy's support for nuclear fission research be reduced by 22% — largely by abandoning work on the Clinch River liquid metal fast breeder reactor — but that there be a 34% increase for research into the uses of coal.

Despite a continued failure to persuade Congress to accept his arguments for killing the Clinch River project, the President is recommending that support for LMFBR research be cut from $615 million to $300 million. In addition, he is also suggesting that research be terminated on the gas cooled fast breeder concept, currently supported at a level of $26 million a year and on the thermal reactor fuel cycle.

Reflecting a growing political interest in coal as a source of energy, the budget recommends coal research be increased from $779 million to $1047 million.

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Oceanic and Atmospheric Administration.

Funds for basic biomedical research at the National Institutes of Health are scheduled to be raised by 6.2% to $1.7 billion. Congress is likely to add a little to this figure, but it will be nowhere near as substantial as in the last decade.

Of the various biomedical research institutes, the National Institute of Environmental Health Sciences is slated for the biggest increase — 15% — and the Institute of General Medical Sciences 6%, while the two largest institutes, the National Cancer Institute and the National Heart Lung and Blood Institute, are planned to grow 0.7% and 3.8%.

In the National Science Foundation, which is planned to increase its basic research budget by 18.8%, special emphasis will be given to supporting research in the physical sciences and engineering. For example, the budget request allows for a 16% increase for physics research, 28% for computer research, and 28% for electrical, computer and systems engineering research.

In contrast, support for biological, behavioural and social sciences is scheduled to grow by only 9.3% — scarcely above the expected level of cost inflation.

Prominent in the NSF's budget request is increased funding for research projects jointly sponsored with industry, a move prefigured in the President's innovation message to Congress last November. For example, support for small business innovation and industrial technology will grow from $7.4 million to $18.2 million, and for industry/university cooperative research from $7 million to $20 million.

The most dramatic manifestation of this new linkage is agreement between the NSF and several major oil companies to jointly sponsor a 10-year, $700 million ocean margin drilling programme (*Nature* 24 January page 321) using the ship Glomar Explorer as a drilling base.

But the Foundation will also be developing a comparable programme of automotive research, which will receive initial federal funding of $20 million in 1981. The estimated costs of $1 billion over ten years will be shared between government and the automotive industry. ☐

BUDGET REQUEST FOR FISCAL YEAR 1981 (in $ million)

Agency	**R&D obligations[1]**			**Basic research obligations**		
	1981 request	**%increase 1980/81**	**% of total**	**1981 request**	**%increase 1980/81**	**% of total**
Defense	16,565	20.2	45.7	523	21.4	10.3
NASA	5,617	9.8[2]	15.6	581	8.3[3]	11.5
Energy	5,106	3.8[4]	14.1	593	13.4	11.7
Health and Human Services	4,011	6.0	11.1	1,840	6.5	36.3
(NIH)				1,704	6.2	22.6
National Science Foundation	1,056	17.7	3.9	952	16.9	18.8
Agriculture	786	5.8	2.2	324	12.1	6.4
Environment Protection Agency	445	7.3	1.2	19	36	0.4
Interior	415	—1.0	1.1	78	2.6	1.5
Commerce	379	2.2	1.0	36	28.6	0.7
Others	1,756	16.0	4.8	127	39.5	2.5
Total	**36,136**	**13**	**100**	**5,074**	**12.0**	**100**

1. Obigations is the amount of money committed to a programme
2. Takes into account extra $300 million requested for 1980
3. Excludes cost of spacecraft
4. Excludes energy research money expected from energy security

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Part of these funds will go to three large demonstration projects for coal liquefaction and gasifaction processes, carried out on a cost-sharing basis with private industry.

$15 million allocated for new UN science fund

THE Carter administration proposes in its budget request to contribute $15 million in 1981 to an interim fund to support scientific activities in the Third World agreed at the UN Conference on Science and Technology for Development (UNCSTD) in Vienna last year. The size of the contribution is less than had initially been hoped for, and some feel this may reflect US unhappiness about the reorganisation of science policy machinery within the UN.

High energy physics keeps its head just above water

DESPITE previous agreement that funding for high energy physics would do no more than keep pace with the general rise in costs, the administration has responded to recent complaints by the physics community and agreed to a 10.5% increase in the budget for 1981, officially representing a 1.5% growth above the predicted 9% inflation rate.

This increase will bring the HEP budget to a total outlay of $350 million in 1981, a sum which includes the final year of funding for Fermilab's energy saver/doubler project, and the initiation of the tevatron colliding beam facility at the same site.

A 9.5% increase is being requested for nuclear physics. In addition to supporting the continued construction of the National Superconducting Cyclotron Laboratory at Michigan State University, this money will also allow construction work to begin on a tandem accelerator system at the Argonne National Laboratory.

Gamma ray observatory preferred to comet mission

PLANS for a gamma ray observatory were given funding preference by the National Aeronautics and Space Administration over a proposal to initiate work on a joint flight to the comets Halley and Tempel 2 because it offers "a more fundamental mission", according to NASA Administrator Dr Robert Frosch.

The administration is asking Congress for $19.9 million to begin work on the observatory in 1981, a project actively canvassed for by space scientists for several years as a successor to earlier gamma ray satellites. The observatory is now scheduled to be launched from the space shuttle in 1985.

Dr Frosch told a press briefing that there was a "reasonably good chance that the much-delayed space shuttle, still officially scheduled for launch this summer, would have its first flight by the end of the year, although there was a likelihood that it would have to be postponed until the first quarter of 1981. In addition to a 1981 budget request $800 million more than originally planned for the shuttle, NASA is also asking Congress for an extra $300 million for the current year to cover unexpected costs, largely the result of problems with fixing insulating tiles to the shuttle's surface.

NIH to reduce training grants

THE National Institutes of Health is to reduce the number of training grants for postdoctoral students in 1981 by 7% from the 1980 level of 10,175. The most significant reduction will be in individual grants, which will drop 33% from 1626 to 1080. Institutional grants will be reduced by 2% to 8390. At the same time the value of stipends will be increased by about 5%, and this comes on top of a 30% increase agreed for 1980. In 1981 stipends will range from $14,040 to $17,892 a year, compared to $10,000 to $13,000 in 1979.

The total value of training grants issued by NIH will be reduced by 7.4%, from $176 million to $163 million. At the same time the administration has agreed to keep the number of new competitive research grants at about 5000.

United States

PCP dioxins found to pose health risks

Following wide debate on the hazards of TCDD, US scientists are now expressing concern about the possible health hazards of low-level exposure to other dioxins, particularly those in the widely-used wood preservative pentachlorophenol (PCP). **David Dickson** reports

SEVEN years ago, Dale Feucht, a dairy farmer in Canton, Ohio, decided to build a new barn for his herd of several hundred cattle. Two months after the animals had moved into their new home, they began to suffer various ailments, from general ill health to a rapid drop in the calf survival rate.

Veterinarians were at a loss to explain or treat a variety of apparently unrelated symptoms, affecting both existing stock and the animals bought to replace those that died. Within a few years, the herd had been decimated, and the last cattle died last summer.

Having eliminated changes in the water or feed supply as the source of the problem, suspicion focused on the one new factor in the environment, the barn. In particular high levels of pentachlorophenol (PCP), used to treat the wood as a preservative, was found in blood samples from the dead animals — assumed to result from the cattle chewing or rubbing the wood.

Mr Feucht is now suing the barn manufacturers, Morton Company. The case, to be heard in Ohio within the next few weeks, is being watched by many large chemical companies.

For PCP is currently one of the most widespread chemicals in domestic use, particularly in demand for its highly effective anti-fungal and anti-bacterial properties. Over 50 million pounds are produced annually by over 250 companies, and world-wide production is more than 100 million pounds.

Recent events, however, have started to fuel public controversy over the long-term, low level effects of dioxins that have so far been largely overshadowed by the more obvious effects of the most toxic dioxin, tetrachlorobenzo-p-dioxin (TCDD), both a contaminant of the herbicide 2, 4, 5-T, and the chemical released in the Séveso accident in 1976.

The toxic properties of PCP itself have been known for some time. Ironically it was the development of oedema in chicks that first warned scientists of the possible dangers of dioxins. Little is known of the physical, chemical and toxicological properties of other dioxins, particular the hexa-, hepta-, and most prevalently in PCP, the octachlorodibenzo-p-dioxin.

So far, no firm action has been taken against PCPs, which remain on the shelves of hardware stores throughout the country merely with warnings about the need for careful use. Timber companies, for example, do not issue warnings with wood that has been treated with PCP.

Two years ago, the Environmental Protection Agency, following evidence of the teratogenicity and feto-toxicity of PCP during animal laboratory tests, announced that it was studying whether tougher regulations were needed. These are now expected to be published later in the year.

In the meantime, the lack of strict regulations meant that the Dow Chemical Company, having introduced a new preservative, Dowicide EC7, in which dioxin levels had been reduced two orders of magnitude by using more expensive production techniques, found the product uncompetitive against rival, non-decontaminated brands. Dowicide EC7 has now virtually disappeared from the market place.

But the political momentum previously lacking may now have arrived. Last year residents near the Lexington Blue Grass army depot in Kentucky started to claim that a high local incidence of leukaemia, as well as various other medical disorders, was the result of exposure to PCP used to preserve ammunition boxes at the depot.

Not only had workers in the depot been exposed to the chemical, but wood from the boxes had been sold off for as little a one dollar a car load. This was used primarily for firewood, but investigators subsequently found a range of other uses, including the construction of porches, barns, chicken coops and pigpens.

Following information collected by a local physician, a preliminary study carried out for the EPA by research workers from the University of Miami confirmed that there appeared to be an abnormally high incidence of leukaemia in the area.

Further data are now being collected by researchers from the National Institute of Occupational Safety and Health. They face a double detective task: first to discover whether the leukaemia incidence is substantiated by epidemiological data: secondly to determine whether, given the other substances at the base heavily used duirng the Vietnam War to store chemical warfare agents, the blame can be placed on the PCP.

But in the wake of the Agent Orange and Séveso incidents, suspicion has been focused on the wood preservative and the contaminants that it contains. And in addition to results of laboratory animal studies, this suspicion is also supported by recent research carried out at the National Institute of Environmental Health Sciences and North Carolina State University into the effects of PCP in cattle feed.

Research workers at the two institutions conducted a study under which five groups of heifer were compared over a 160-day period. One group was given a feed containing analytical PCP prepared in the laboratory, a second fed with technical PCP containing the dibenzodioxin and dibensofuran contaminants found in commercial preparations. Two groups were given a mixture of the two. The fifth group was used for control.

The results of the research, which are soon to be published, indicate a number of dose-related effects in the heifers exposed to the contaminated PCP, compared to the controls or those exposed to the analytical PCP. These included a decrease in body weight and feed efficiency, as well as progressive anaemia, and at the end of the test the researchers found a number of unexpected lesions in cattle using the contaminated feed, in particular in the mucosa of the urinary bladder through which the PCP would have been secreted.

"The results of our study show clearly that the toxicity of PCP in cattle is primarily attributable to its contamination with toxic impurities, either the dibenzodioxins or the dibenzofurans," says Dr Eugene McConnell, head of the Comparative Pathology Section of NIEHS and principal investigator of the study.

Which of the two is the culprit has not been demonstrated, but the North Carolina results are broadly compatible with evidence from low level exposure of monkeys to dioxins currently being carried out by Dr James Allen at the University of Wisconsin. Despite its lack of conclusiveness, such scientific evidence is likely to play an important role in forthcoming court cases.

William Brown, the attorney representing Mr Feucht, said last week that he was already dealing with two other PCP-related claims, one from a family that had built a house from PCP-treated timber supplied by a do-it-yourself construction company, the other from a farmer claiming disability after handling PCP-treated posts.

Mr Brown added that he had been contacted by others with similar complaints. A number of other court cases are pending in Michigan. "Everyone is watching this case very closely," says Mr Brown, pointing to the widespread use of PCP-treated timber, not only in homes and farm buildings, but also for railway sleepers and telegraph poles. "I really think it is the tip of the iceberg." □

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Soviet Union

Sakharov arrest threatens East-West scientific exchange

Vera Rich reports on the reactions of the scientific community in the West to the arrest of Dr Andrei Sakharov (right) in Moscow last week and his subsequent internal exile

BANISHING Dr Andrei Sakharov, physicist and leader of the human rights movement in the Soviet Union, to Gor'kii is not the way to promote international scientific exchange. This is the clear consensus of the western scientific community.

Referring to Sakharov's "internal exile" as "a shocking" and "a blatantly punitive" act, Dr Philip Handler, President of the US National Academy of Sciences, noted that "in recent years the scientists of both nations have carefully built structures of communication and good will", and that the move against Sakharov "can only be regarded as a challenge to further cooperation and an act of deliberate ill will. . . . I find it difficult to imagine scientific exchange continuing in the spirit we had created heretofore."

"The exile of Professor Sakharov", said Kenneth Boulding, Chairman of the Board of Directors of the American Association for the Advancement of Science, and Frederick Mosteller, President of the AAAS, "deprives the people of the Soviet Union and the world of a brilliant voice in support of mutual understanding and the defense of human freedom". His banishment, according to 25 US Nobel laureates, who sent their own personal telegram to Brezhev "will silence an eloquent voice of wisdom desperately needed in our troubled world".

For the US Committee of Concerned Scientists, Drs Max Gottesman and Mark Kac, expressed their concern that the move against Sakharov coming "as the aftermath of Afghanistan" might "portend a change which threatens the continuance of cultural and scientific relations between our two countries." Telegrams from the French Academie des Sciences and Société des Physiciens expressed similar shock and concern.

In London, the "President and Officers" of the Institute of Physics, expressed "shock and dismay" fearing that "this action . . . will damage both Soviet physics and the relations between Soviet and British physicists". The Royal Society, with its usual diplomacy, expressed the view that "voices sound louder through doors that are open than through those that are locked".

This is not the first time that western colleagues have felt obliged to leap to Sakharov's defence. In September, 1973, an ominous *Pravda* article, entitled "To be a Russian scientist means to be a patriot" was widely seen as a first move towards expelling Sakharov from the Academy of Sciences. No expulsion in fact took place, perhaps due to the storm of foreign protests the article provoked, but also to the reluctance of the Soviet Academy to permit undue government and Party meddling in its internal affairs. (The Academy is the last remaining Soviet body to have elections by secret ballot with a plurality of candidates). A short while later, when a similar attempt was made to deprive Veniamin Levich, the Jewish "refusnik" of his "Corresponding" (i.e. Associate) membership of the Academy, the attempt failed, his son Aleksandr told *Nature*, "because even some members who were quite anti-Semitic had decided to vote against the motion, feeling that their own position was threatened by any attack on the Academy".

Perhaps as a pre-emptive measure against such academic solidarity, Soviet "explanations" of the Sakharov affair have stressed how much he is out of step with the ideal of a Soviet scientist. The initial allegation (in *Izvestiya*) was that he had abandoned science for "the political walk of life" following a crisis in his own creativity. (No mention was made of his sudden dismissal from his government job — following the *samizdat* publication in 1968 of his "thoughts on Progress, Coexistence and Intellectual Freedom").

This approach, however, was negated when the western media stressed that Sakharov had been arrested on his way to a physics seminar at the Academy of Sciences — and by confirmation from close friends that he was still, in spite of the demands on his time made by his human rights activities, keeping up his theoretical work on physics, though with little hope of publication. Indeed, the *Izvestiya* accusation, that Sakharov was being "used as a channel for passing on important Soviet secrets to the . . . imperialist powers" was incongruous with their allegation that he had abandoned science.

The next stage came with an attack on Sakharov by individual members of the Academy. Academician Evgenii Fedorov spoke of the committment of Soviet scientists to "social work, the struggle for peace, cooperation with our colleagues abroad in science and technology" (almost echoing the title of Sakharov's first essay!). A "large community" of Soviet scientists, he said, are "fed up with Sakharov's remarks and his general attitute . . . contrary to everything for which Soviet people, including representatives of Soviet science, have fought". Academician Mark Mitin spoke of the "humanitarian" action of the Soviet government, which in spite of Sakharov's slanders of the Soviet way of life had simply deprived him of his state honours and sent him out of the capital. Nevertheless, Mitin observed unctuously, "as a member of the same Academy of Sciences I cannot but feel sorry about the downfall of this man."

Some, it would seem, felt more than mere sorrow. The resignation of Vladimir Kirillin from his posts as Deputy Prime Minister and head of the State Committee for Science and Technology was almost certainly connected with the Sakharov affair — though speculations vary as to whether Kirillin resigned voluntarily in protest or was manoeuvred into a situation where resignation became inevitable. Of recent years he has been a well known visitor to Britain to negotiate extensions to exchange agreements in technology and trade. (The next round of talks, due in London in May, will almost certainly now be at least postponed).

During the past few months, there have been a number of indications of conflict between the State committee (which comes directly under the government) and the Academy, which still shows a remarkable independence of spirit, paticularly where its own autonomy is concerned.

Although the move against Sakharov must be seen against a whole wave of repressions against a wide spectrum of human rights activists over the past few months, the fact that the authorities felt able to renew their challenge to the Academy may suggest that the strength of the Academy has somewhat waned in comparison with 1973. Certainly, it is Dzherman Gvishiani, a representative of the State Committee who will lead the Soviet delegation to next month's Hamburg Forum — an event which, incidentally is still going ahead, although as one of the organisers told *Nature* "current political tensions will naturally be reflected there in some way". □

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Soviet Union

Fire threatened fast reactor cooling system, says unofficial report

"WE have no accidents which would give rise to anxiety among people and set them aginst the development of nuclear power engineering," Andronik Petrosyants, chairman of the State Committee for the Utilisation of Atomic Energy, told the press recently.

This, however, is not the opinion of the townspeople of Sverdlovsk, in the Urals. A clandestine report which has just reached the West gives a vivid description of the alarm caused by a fire at the Beloyarsk nuclear power station some 60 km away.

The author, who identifies himself only as "N.N" appears to have inside knowledge of the workings of the power station. He alleges that during New Year's Eve celebrations in 1978/79, a fire started in the "machine hall". Several firefighters lost their lives in combatting the blaze. It was not brought under control until "everything that could possibly burn was completely burned up."

"N.N" admits that there was no immediate risk of the reactor exploding; however, the reactor cooling system was, he says, threatened. The Beloyarsk station has a fast-reactor, and the primary circuit of the heat-exchanger contains highly radioactive molten sodium at 500°. A rupture in the primary circuit would cause an explosion scattering radioactive material in a cloud like that produced by the 1957 Kyshtym disaster.

"N.N" says that the threat of a similar cloud from Beloyarsk was sufficiently real for the authorities to provide special trains and buses, standing by to evacuate, if necessary, the settlement of Zarechnyi close to the plant.

Following the fire, says "N.N", a KGB team from Moscow, led by a General, came down to investigate. Their findings remain, he says, unknown, but the local population feel that a significant part was played by the fact that "the shift was drunk, like almost everyone else in the Soviet Union that night." No official allusion has been made so far to any fire at Beloyarsk.

Like the 1973 fire at the Shevchenko fast breeder, which was due to a rupture in the sodium circuit, the Beloyarsk fire was not technically a "nuclear" disaster. According to Academician Anatolii Aleksandrov, President of the Soviet Academy of Sciences and himself an experienced nuclear engineer, fully half the funding of nuclear power stations is spent on safety.

This however, N.N's report suggests, is insufficient; the human factor also must be taken into account. **Vera Rich**

High energy physics

End of the physics phoney war

Competition for the prize of the intermediate vector boson is leading to doubts about the future of CERN, the European centre for subnuclear physics. But the real competition may be trans-Atlantic. **Robert Walgate** reports

IN a few weeks' time, the European Committee for Future Accelerators will be holding an extraordinary meeting to discuss what amounts to a perceived competition between West Germany and CERN. ECFA, which has no constitutional power but which represents the views of an influential body of European physicists, is thus cautiously falling in line with Italy, which first shed doubt on Germany's intentions.

The Chairman of ECFA, French physicist Professor Marcel Vivargent, was recently quoted in an Italian newspaper as saying that Italian efforts to clarify CERN's future programme "merits the best attention" and is supported by many European physicists. (In late December, the Italian delegation to CERN walked out of a meeting of CERN Council in a protest at "lack of sufficient discussion" of CERN's future, and allusion to potential German competition.)

Vivargent told *Nature* that he understood the Italian position, as a relatively small nation relying on CERN: "they want CERN to remian the top lab in Europe". He also said that he understood the German desire to find a future for its lab, DESY. The problem, which had been neglected by CERN Council, was to mesh the two objectives together.

Strictly ECFA will be discussing 'complementarity' between two accelerator proposals; HERA for the German laboratory DESY near Hamburg, and LEP for CERN. HERA is designed ultimately to collide 30 GeV electrons with 820 GeV protons and shed light on interquark forces, and perhaps the structure of the quark. (It is equivalent to a 52 TeV electron linac firing at a stationary target, giving a 'spatial' resolution of some 100,000th part of the proton radius.)

But the first part of HERA, according to its coordinator Bjorn Wiik, will collide 35 GeV electrons with equal energy positrons. It will operate in this mode for four years before the proton ring is complete. "Our proton ring will be by far the biggest superconducting effort in Europe" explains Wiik "so we have allowed a long lead time to build it". According to present targets, the electron phase of HERA will be ready in 1985, and the protons in 1989.

LEP, on the other hand, is dedicated to the collision of electrons and positrons at beam energies between 22 and 130 GeV. Protons do not come into the proposal, although the proximity of the CERN complex of proton machines, up to the 400 GeV SPS, make the use of protons a possibility.

The gem in this energy range is the Z^0, which at 90 GeV would be the heaviest particle yet and a key element in the unified theory of the weak and electromagnetic interactions. In the theory, due to Abdus Salam and Steven Weinberg, the Z^0 plays the role of the photon in the weak interaction (which causes nuclear decay and limits the output of the stars).

The Z^0 (or neutral intermediate vector boson) is mesmerising physicists like Moby Dick mesmerised Captain Ahab. LEP would create it (if it exists) with 45 GeV colliding beams. So would HERA, if it could be stretched up to that energy; and since approval for LEP is unlikely to come before mid-1981, and construction (at a constant CERN budget) is predicted to take seven years, there is a possibility — it is argued — that HERA would have snatched the Z^0 four years before LEP.

The question is actually more complicated, because CERN is likely to find the particle in 1981 — but not with LEP. CERN is building a proton-antiproton collider which will produce 270 GeV on 270 GeV collisions towards the end of 1981, with a view to creating a few Z^0s. But it will not produce many — opinions

Machines which might compete for the Z^0

Device	Projected cost	Circumference	Earliest likely physics	Beam energy	Luminosity* +
LEP	$640m	30.6 km	1989+ + +	22-130 GeV**	10^{32}cm^{-2}s^{-1}
HERA	$167m + +	6.5 km	1985 (electrons)	35 GeV	10^{32}
SPC	$60m	1.8 km*	1984	50 GeV	10^{30}
antiprotons	(budgeted)	6.8 km +	1981	270 GeV	10^{30}* + *

* in two loops

+ in the SPS ring

** main option 86 GeV

+ + electrons only; with protons double this

* + event rate per unit collision cross-section at peak energy

* + * fraction of events producing Z^0 some 30 to 50 times lower

+ + + could be earlier if large fraction of present research programme cut

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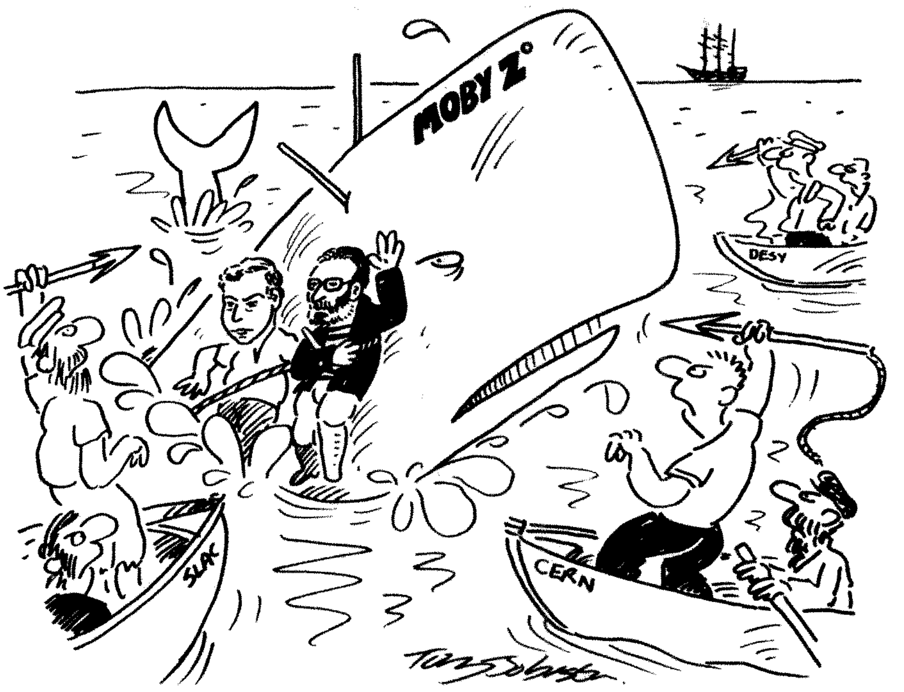

differ, but perhaps some four orders of magnitude fewer than LEP — and those will come among a great deal of unwanted matter (from the spare quarks in the proton and antiproton).

The goal is to produce a lot of clean Z^{0}s, so that they can be studied in detail. If HERA stole LEP's thunder, questions would be asked about the value of LEP. And since CERN has argued that it can run down a number of its facilities, such as the intersecting storage rings, to leave room in its present budget to build LEP, it may find that member states are asking it to run down — without LEP.

But Wiik, HERA's builder, quashes the speculation. "At 45 GeV, the synchrotron radiation [X- and gamma-rays emitted as the electrons turn corners] in HERA might be completely prohibitive" he told *Nature*. "And we'd need a large investment in extra cavities — something we haven't budgeted for".

The principle limitation on the energy of electron machines is the amount of radiofrequency power that can be pumped in to keep the electrons in their orbits, to compensate for synchrotron emission. The field of the bending magnets is usually low, and not a limitation (contrasting with proton machines).

"Furthermore" says Wiik "the geometry of the machine is not optimised for 45 GeV". DESY has designed in long straight sections (which increases corner curvature and hence synchrotron radiation) to allow polarisation of the electrons for electron–proton studies. Wiik could not give a figure for the power consumption that HERA would demand at 45 GeV (although it increases from 8 to 13 MW from 30 to 35 GeV) but added that "I don't know even if we would have room to put the extra cavities in". According to one observer, superconducting, cavities would be necessary, and these are not likely to be available by 1985.

The HERA design is almost complete; and later this year there will be an application to Bonn for the DESY budget to be increased by some DM60 million a year from its present DM150 million. Over 10 years, this would be expected to pay for both phases of the accelerator; there will not be a formal application to build HERA. The CERN subscription would not be affected either; "that money comes from a different pot" said a DESY spokesman.

Competition for LEP, however, may arise from an entirely different quarter: the United States.

Over on the West Coast, at the Stanford Linear Accelerator Center, Professor Burton Richter has a plan for a cheap "Z^0 factory". At an estimated $60 million ("plus or minus 25%" says Richter) the 'single pass collider' would undercut first phase HERA costs by a factor of three, and LEP (main 86 GeV version) by a factor of ten.

Richter's device gambles on the presence of the Z^0 (a fair bet) and makes use of the 90 GeV resonance to avoid the need for large and costly storage rings. Thus Richter turns the tables. His small storage ring SPEAR won him the Nobel prize and set the current fashion for storage ring physics (LEP is basically a scaled-up version of SPEAR).

Since the cross-section at the resonance will be very high, he reasoned, a single collision between one bunch of electrons and one of positrons should be enough to produce a reasonable number of events. So he constructed a plan around the SLAC one-mile electron linac that would do the job. It involves increasing the energy of the accelerator to 50 GeV; and accelerating an intense pulse of positrons down the linac followed, 20m behind, by one of electrons. At the end of the accelerator these pulses are wheeled around a radius of 300m one to the left and one to the right, and brought into collision.

Richter is very confident that the machine can be built, despite very close tolerances. SLAC is pursuing a design study and will make a request in March for full funding. If the application went smoothly, the SPC could begin building in October 1981, "but I give that no better than evens" said Richter. In the long term he considers political prospects for the collider to be "very good. Everyone is enthusiastic about it".

Since the project is small enough to avoid the need for an externally imposed managing architect (the choice of which delayed PEP, SLAC's medium energy electron ring, due to start in March, by nine months), the SPC could be built in two and a half to three years, thinks Richter.

The technical obstacles are: accelerator improvements, where SLAC is putting in its largest theoretical effort (it will be necessary to align the accelerator components to 0.3mm to stop the long pulses slewing around, tail to the side) increasing the brightness of the positron source by a factor of two (a design exists for a factor ten, but engineering R&D is necessary); "damping" the energy spread of the positrons from the source; synchrotron radiation in the 300 m bends; and a final focus of the beam spots to 2 μm.

This latter compares with the 100 μm × 1000 μm spot in SPEAR, or the 90m diameter spot emerging from the linac. But the exercise is one of geometrical optics, particularly getting rid of the 'chromatic' aberrations present in the beam transport system given a beam momentum spread of 0.5% and this is designed in principle.

Finally the question is the number of Z^0 that the SPC could produce. "A million a year" says Richter — quite enough to learn a lot of physics. LEP would have 100 times more, and eight experimental regions, but the SPC ought to be able to detect all but the rarest decay modes of the particle.

This leaves LEP with the rare decay modes, and its higher peak energy, which with its main (86 GeV on 86 GeV) version should produce the charged companions to the Z^0, the W$^+$ and W$^-$, which are produced most efficiently in pairs.

This is good physics, but there are some doubts about how efficiently a storage ring could work at this energy. Further the power consumption will be extremely high (some 250 MW at peak power). LEP will consume an average 750 GW hours per year for 3,000 hours of experiments at 85 GeV raising doubts about running costs against electricity costs in the late 1980s and 1990s.

Although physicists are in wide agreement that LEP is by far the best possible machine to explore the physics of the Z^0 and the Ws, and to search out what lies beyond Weinberg and Salam, the machine is strangely beginning to look like a Rolls Royce against competition of a Model-T Ford which, nevertheless, gets you where you want to be. □

NEWS IN BRIEF

Livermore earthquake raises safety concerns

A debate about the safety of nuclear research facilities at the University of California's Lawrence Livermore Laboratories has been rekindled by an earthquake which hit the laboratory last Thursday causing minor injuries to almost 30 employees, and leakage in a storage tank containing tritium contaminated water. According to the laboratory, no radioactive material was released into the atmosphere or the environment as a result of the accident and key research buildings containing plutonium and tritium sources were undamaged. However, the laboratory added that a 30,000 gallon tank containing 50 microcuries/cc of tritium had leaked about 50 gallons into an asphalt basin on the ground, although none had escaped from the containment building.

The earthquake is also reported to have caused $1 million worth of damage to the Shiva facility. The bolts, holding down the metal frame holding the 132 ft facility, sheared. Other damage at the laboratory included the displacement of filing cabinets and office trailers which were knocked off their stands. Last year, the earthquake hazards faced by the laboratory became a major source of controversy when local groups seeking to curtail nuclear weapons research at the laboratory claimed that earthquake damage could lead to a major leak of radioactivity. Last week's earthquake which measured 5.5 on the Richter scale and was centered 10 miles from Livermore was one of the severest to hit the San Francisco region since the major earthquake of 1906.

Plan to dump toxic wastes in Third World

A number of US waste disposal companies are drawing up plans to dispose of toxic wastes in Third World countries as a result of the imminent introduction of strict regulations covering the dumping of such substances by the Environmental Protection Agency.

One Colorado company was reported last week to have plans to open waste recycling plants in three foreign countries, thought to be Sierra Leone, Liberia and Chile. Officials from the company, Nedlog Technology Group Inc., said that the plants are planned to extract metals such as copper, cadmium and zinc from industrial wastes.

Under new regulations it might prove impossible to dispose of wastes from this process in the US. The company was therefore "looking overseas to find countries that need foreign exchange and jobs and have the plant sites," said company vice-president James R. Wolfe.

State Department officials, however, are worried that any such moves by US corporations could generate a backlash in the developing countries, which might decide jointly to "condemn the US for dumping its wastes in the black man's backyard".

Deutch to resign from Energy Department

THE US Department of Energy confirmed last week that Dr John Deutch, previously director of the Office of Energy Research, and currently Under-Secretary of Energy — the third ranking position in the department — had submitted his resignation to the White House.

During his nomination hearings before the US Senate in 1977, Deutch made it clear that he intended to remain in Washington only for a limited period of time before returning to his position as professor of chemistry at the Massachusetts Institute of Technology.

However, speculation about Dr Deutch's future plans have been raised by reports that he may join President Carter's re-election campaign as an energy adviser and that White House aides are said to be increasingly disappointed with the political performance of Energy Secretary Charles Duncan. In particular, they are critical of his failure to "politicise" the department, for example by not taking a strong public stand criticising Senator Edward Kennedy's opposition to the decontrol of oil prices.

Nobel Prize changes Pakistan's science

Science in Pakistan has received a major boost after the award of the 1979 Nobel Prize for phycics to Abdus Salam. Pakistan's President, General Muhammed Zia-ul-Haq has publicly promised to allocate 1% of the country's GNP for scientific and technological research in accordance with suggestions made by Salam. This would mean an expenditure of $100 million annually instead of only $20 million at present.

The President has also agreed to give serious consideration to another far reaching proposal made by Salam at the Special Convocation held at the University of Islamabad during his recent two week visit to Pakistan. That is to make science, like religion, a compulsory subject at all levels of education. Since Salam's visit, scientists have been made exempt from the general ban on travel abroad.

Azim Kidwai

French cracks cause new nuclear start up delay

THE French Ministry of Industry announced last week that it would postpone by "several weeks" the scheduled start up of the troubled nuclear reactors, Gravelines I and Tricastin I. Cracks in the tubular base plates of the heat exchangers in the primary cooling circuits have already caused delays because of strike action by the trade unions involved who have demanded additional safety measures. The new delay will permit further tests to be made on equipment that has been desgined to follow automatically the evolution of the cracks during the lifetime of the reactors. Preliminary tests of the monitoring equipment by the manufacturer, Framatome, have been inconclusive because they have been done on surplus plates, originally scheduled for export to Iran, that have no cracks.

In the meantime, a petition campaign launched last June by 22 organisations for "an alternative energy policy and for a democratic debate on energy" has received 500,000 signatures.

Ariane given to private industry

THE council of the European Space Agency made final last week the French proposal to transfer the Agency's launch capability to the private firm Ariane-Espace. Ariane-Espace will be responsible for production and launching of all future ESA satellites. It will charge the Agency the following prices for various kinds of launches: 175mFFr for a launch requiring full Ariane power, 150mFFr for a satellite launch requiring only part power, and 95mFFr for a satellite that can be launched along with another in a double payload. The director-general of ESA will engage in discussions with the directors of Ariane-Espace to elaborate the details of the arrangement.

The ESA council also voted approval of the delayed 1980 budget and made the Earthnet programme of earth and meteorological satellite observations part of the official ESA budget. Earthnet had been previously funded on a voluntary basis by member states. The council also voted the funds for the Spacelab programme. In a related development, Switzerland, which is not an ESA member state, voted a sum of 7mFFr to participate in future improvements of the Ariane launcher.

Correction

In *Nature* 17 January, page 237, the statement by the Scientific and Medical Committee for Soviet Jewry should read: "not only should the Hamburg meeting include on its agenda the question of the freedom of movement of scientists, also any scientific body that may arise from the meeting must ensure that a clause dealing with this problem is written into its constitution".

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China: where the new and the historical materialism interact

Although scientists in China are being forced into a new role by rapid changes in Chinese life and closer contacts with the West, they are still guided by Marxist philosophy and the principle of social relevance. In the first of three articles on developments in Chinese science policy over the past year, **Tong B. Tang**, describes the organisation of research. In future articles, he will discuss higher education and the popularisation of science.

SCIENCE in China is growing increasingly quickly. It is based on ancient scientific heritage, the tradition developed under Marxism over the past 30-60 years, and the critical adoption of western methods and ideas. The importance of the last factor, however, has yet to be assessed. Although China stresses that its science will take a different path from that of western countries, its science and technology now seem poised to fill the economic and military roles which they have long occupied in industrialised countries.

China needs to promote the national economy and to deter what it calls international and regional hegemonists with greater help from the modernisation of science. Hence, the direction and prospect of its science are bound to be of profound interest, not only to scientists and science historians, but for the world.

Most scientific research is carried out at the 112 institutes of the Academia Sinica and at the 600 or more universities. The Academia, which celebrated its 30th anniversary in November 1979, is the designated "comprehensive centre of research into the natural sciences".

The entire pure and applied science budget is administered by the State Scientific and Technological Commission with Vice-Premier Fang Yi in charge. The Commission's preferences for different disciplines basically follow the National Plan for the Development of Science and Technology, a document of many volumes which was collectively agreed at the All-Nation Science Conference in March 1978 (*Nature*, 6 April 1978, page 482). Briefly the orientation is towards agronomy and applied physics. The Plan was slightly modified when the three years of 1979-81 were recognised as an adjustment period for the national economy, and a conference was convened by the Commission in November 1979 to discuss the implementation of these modifications, particularly in the area of science and technology transfer.

The Commission allocates research responsibilities and funds to the Academia or the Education Ministry, and then successively to institutes or universities, sections or faculties, each represented by 'academic committees', and finally research groups. The Academia, nominally led also by Fang Yi, has Li Chang as its First Vice-President, and the Minister of Education is now Jiang Nanxiang. Key installations may be operated directly by the Academia or the Ministry or both. In addition, other ministries may support researchers whose work is of direct relevance to agriculture, forestry or industry. Some of them run their own chains of research institutes and training colleges. However, the infrastructure that couples the (long-term) activities of institutes and universities to research carried out at scientific farms and research and development at factories appears weak; one indicator is the relatively long delay that sometimes occurs between completion of a project and the application of its result in production.

Giant propaganda poster stresses the political context of modernisation

Vice Premier Fang Yi, in charge of China's science budget and head of the Academia Sinica, meets students in Beijing

Most research projects are assigned from above, but occasionally a research group submits a proposal to the academic committee of its section or department, whose complete programme will be reviewed by the institute or university. But it is unusual for a research group to branch out in its own research areas. This contrasts with western universities but probably resembles more the situation in government and most industrial laboratories. Last November, however, the Academia passed a resolution to give institutes the freedom of securing external funding by taking on contract work. The research unit will be able, after making sure that its state-assigned projects will be

Tong B. Tang is a research fellow with the physics and chemistry of solids group at the Cavendish Laboratory, Cambridge

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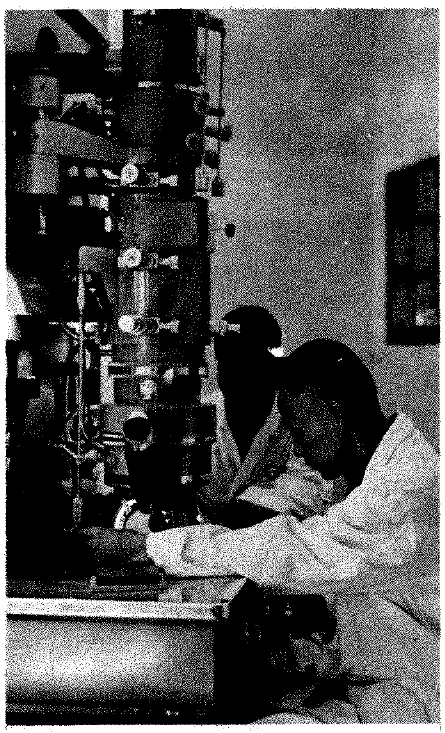
completed on time, work on problems offered by a factory. Its time and expenses will be repaid by the factory either in one lump sum or as a percentage on the sale profit of the product concerned. It does not have to hand back to the state any net income that may result, but this procedure is said to be temporary only.

That the applied sciences continue to dominate in China is a reflection of the conviction that science receives its impetus from social needs: "from the very beginning the origin and development of the sciences have been determined by production". However the "socialist newborn-thing" of "inviting in" workers and peasants with practical experience to participate in experiments, and to a lesser extent that of scientists "going out" to work with the masses have largely gone. Now more 'pure' research is being encouraged. For example, two groups, one in Beijing, the other in Guangzhou, have started work on the detection of gravitational waves, and an observatory is planned near Kuming to carry out a comprehensive survey of the southern sky. More well known, perhaps, is the construction of a 50 GeV proton synchrotron at Beijing (the 'BPS').

Opinion is divided on the social relevance of going in for high energy physics in a big way, especially among guest advisors such as the Nobel prize winners Chen-Ning Yang and Tsung-Dao Lee. But Li Chang explained at the All-Nation Science Conference that particle physics, being a frontier subject, would play a leading role by stimulating advances in instrument technology and other fields. (The other two 'frontier sciences' which have been identified in a National Conference on Basic Sciences Planning held in October 1977 are astrophysics and cosmology, and the origin of life.) Fang Yi recently reiterated: "We consider basic research fundamental to all scientific progress".

Unfortunately, at present there is probably much duplication of applied research effort, on, for example, metal oxide semiconductor interfaces, stacking faults on oxidised silicon and other topics related to integrated circuit technology, rare earth metals as catalysts in petroleum refining, and lasers. All of these are among the priorities specified in the 1978-85 development plan. However, the nationwide coordination of research and resources and the exchange of results are hampered by bureaucratic barriers between ministries and administrative bodies, although the bureaucracy is no worse than in the West.


Worker-technicians Tsao Mu-Chuan and Chang Shih, working with China's first electron microscope

The Chinese are also discussing the relationship between central planning and local initiative. There is a trend for political authorities at the provincial or municipal level to take a greater part in the administration of science. Provided that democratic centralism is not eroded, and that the relationship of local autonomy to central control follows the changing situation, a limited devolution should make science more responsive to local needs without hindering the fulfilment of a unified state plan. Less tangible, though, are certain ideas on a 'mixed' system for research management, namely that science grows most quickly when it is also partly based on 'market economy' principles.

Many measures are still of a tentative nature. With some problems a variety of different solutions are currently being explored; a common solution will be nationally implemented only after an appraisal or reappraisal of all relevant experience. Up to the winter of 1978-79, policy statements were inclined to be contentiously forthright, for example, those concerning material incentives, learning from the West, and the professionalisation of education. Some of them were more "liberal" than in the years immediately before the Cultural Revolution, even than in the mid-1950s. Since spring of last year this tendency has been reversed. For instance, there has been more propaganda in the media and on public displays emphasising political work, revolutionary heroism, self-reliance and the danger of examination worship.

Western scientists in general and those of the social disciplines in particular are enthusiastic about the bourgeoning academic exchanges with China; about these more will be said in the next article. The Chinese welcome these developments but have firmly concluded that there is no question of importing wholesale modernisation or science and technology. The current slogan concerning foreign technology is, "use, learn, then modify, and finally invent". In April last year a meeting of academics in Beijing urged that Chinese science should retain its origina character. At the First Nationa Symposium on the Science of Science in August 1979, Li Chang reaffirmed that China will nurture a modern science, different from western science, by integrating the latter with the "dialectics of nature". Some Chinese have claimed that the dominant methodology endorsed by western science, while materialistic, is "metaphysical". This means that it contradicts the dialectical laws of thought, with its implicit assumptions of irreducible isolates and unitary identities.

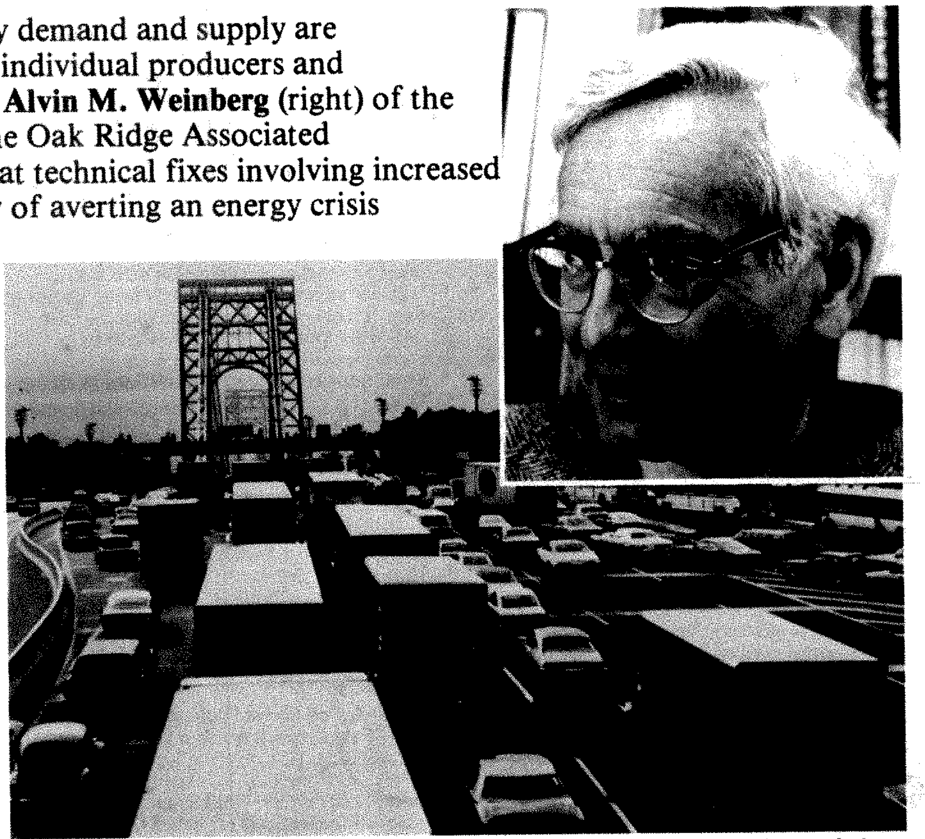
Many events confirm that the prominent features of Chinese science in Mao's era have remained intact and are being further enriched. The Chinese understanding of science (and arts) as orientated towards man's struggle in economic production comes from Marxist historical materialism. Efforts to combine the modern and the traditional, initiated as part of Mao's policy of "walking on two legs", continue. For instance, the Peking College of Traditional Medicine is among the five key medical schools named by the Education Ministry, and traditional medicine is on the (essentially standardised) curricula of all fourth-year students training for western clinical practice or research. Philosophical arguments based on Marxism retain a respected guiding position in scientific controversies. For example in particle physics the quark model is overwhelmingly preferred to the bootstrap hypothesis of hadrons, and the high-sounding applications of catastrophe theory to human phenomena are generally ridiculed.

Further, guided by Mao's conceptions of "one divides into two" and the transformation of opposites, the Chinese want science to "change wastes into wealth and turn harmful into beneficial" in all agricultural and industrial processes. Lastly, to achieve modernisation and a healthy socialisation of science, the Party pledges to mobilise the whole people, in accordance with the dictum that "the mass movement is necessary in all work. Things won't go without the mass movement".

Nevertheless, in its quest for rapid modernisation, the Party has broadened the political base of decision-making and given more scope to the initiative of experts. Senior scientists now join forces with state officials to administer science and education. The press has devoted considerable space to polemics on democracy in academic discussions, or "academic freedom", meaning that the minority can sometimes be right and should always have the right to disagree. Indeed, the frustration of scientists may be understandable especially if it arises from the indifference or the dogmatism of bureaucrats. Still, the leadership will not want greater freedom to result in wasteful competition, an erosion of planned research or the ascent of deviationists and technocrats. ☐

Energy: the need for technical fixes

Model-based predictions of energy demand and supply are unreliable, since the behaviour of individual producers and consumers cannot be guaranteed. **Alvin M. Weinberg** (right) of the Institute for Energy Analysis at the Oak Ridge Associated Universities, Tennessee, argues that technical fixes involving increased supply offer the most reliable way of averting an energy crisis



Increased oil prices have not deterred US consumers, so Carter has chosen to develop synfuels

I first became acquainted with energy demand modelling when I worked for the Federal Energy Administration in 1974. President Nixon had announced Project Independece: the United States was to import no oil after 1980. The FEA's job was to figure out how to reach this goal, or at least some goal that was consistent in spirit if not in fact with the President's announcement. For this purpose, it set up the most elaborate model the budding art of energy modelling had yet seen. Many millions of dollars were chewed up on two CDC 7500s; their neatly printed graphs showed how energy would be used and how it would be supplied in the United States through the year 1985.

A prime question was the price level of imported oil at which one could expect domestic supply to be so enhanced, and demand so dampened, as to reach the goal of self-sufficiency. The computers gave a fairly clear answer. At $16 per barrel, $6 per barrel higher than the 1974 OPEC price (at 1979 values), oil imports could fall to zero by 1985, provided the government encouraged accelerated production of domestic oil. The model predicted that, even without government intervention, US imports in 1980 would be only about 6 million barrels per day, falling to about 5 million barrels per day in 1985. As things turned out, the US imported more than 8 million barrels per day in 1979 even with the price of imported oil a good bit higher than $16 per barrel.

Looking back, it is apparent that the FEA model had overestimated the demand elasticity for liquid fuel — the percentage decrease in demand for a given increase in price. The output of the entire model depended critically on the value of this elasticity. Demand elasticities are notoriously uncertain: those used in various models differ by a factor of ten!

Last October, I (along with 200 energy modellers) attended the Dublin meeting on Energy Systems Analysis sponsored by the European Economic Community. The EEC is sponsoring a "super" model that encompasses the entire community; with sub-models applied to each country. At the Dublin meeting the modellers could compare the results of application of the EEC methods to each country.

This effort is of extraordinary scope. Yet, as the modellers themselves conceded, one can hardly depend on its predictions. Unexpected things happen. The Shah is overthrown; people simply don't react the way the models assume they should.

The moral is clear: policymakers must treat models warily. This is hardly an original view. Yet it seems that the modellers in the United States have had remarkable success with the opinion-makers if not, as yet, with the policymakers.

Most of the more recent models tend to stress reduction in demand rather than increase in supply. This viewpoint is promulgated in the recent report of the Harvard Energy Project, *Energy Future*. The book has already sold more than 100,000 copies. It has been widely acclaimed in the American press as containing both the best appraisal of the energy problem, and the best strategy for its resolution.

To meet the US demand in the late 1980s, the Harvard study estimates that 8 million daily barrels of oil equivalent (mboe) can be saved by conservation, and 3 mboe by widespread use of solar energy. "Conventional" supply, meaning coal and nuclear, is increased by only 5 mboe. These numbers are to be compared with the 37 mboe equivalent total US demand in 1977. Conservation and solar energy, by the late 1980s, are supposed to take care of almost one-third as much energy as was supplied by all energy sources in 1977. Although the Harvard study properly warns that conservation and a transition to 'Solar America' depend on innumerable decisions made by countless people, all conserving energy or installing their own solar devices, it leaves the impression that here was a true, and ideologically acceptable, path to energy salvation.

By contrast, Jimmy Carter's response to the energy situation last June was, to a remarkable degree, supply-oriented: spend $80 thousand million for several million barrels per day of synfuel. The President chose to put most of his money on a technical fix: synfuels. How much of the President's proposal will survive in Congress will depend on how much political pressure is generated by those who believe conservation and solar energy come painlessly.

I doubt that the President and his advisers weighed the uncertainties in predictions of demand when they decided to go for increased supply. But I believe their instinct was right. Technical fixes, especially those involving increased supply, require decisions by relatively few people. They finesse the impossibility of knowing how and how quickly 220 million people will react to a change in price.

Obviously the proper course is to increase supply as well as to conserve. This piece of common wisdom seems to be ignored by some of our noisier conservationists. Despite opposition from this segment of the energy community, I hope the President's commitment to augmenting supply will receive the public support it needs to survive in Congress. ☐

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CORRESPONDENCE

French nuclear cracks pose no danger

SIR, — The article entitled "France faces a cold winter" (29 November, page 436), states that: "Although these cracks are not dangerous at this stage, Framatome experiments show that they tend to enlarge under the temperature transients of normal operating conditions, leading to possible component failure within four to six years, a failure which clearly would have catastrophic consequences involving the total loss of primary coolant."

And further that: "The only other possibility is permanent shutdown after five years" and: "These were the facts made public by both government and unions."

We regret that *Nature* did not consult the manufacturer of the components on which the cracks were found. The time limit of four to six years mentioned in your article (the currently accepted figure is six to eight years) concerns the minimum time for the cracks to fully penetrate the 10 to 16 mm thick stainless steel cladding which separates the reactor coolant from the component perse, and *not* the minimum time for component failure. Moreover this minimum time results from calculations based on the most pessimistic assumptions derived from experimental evidence. Similarly pessimistic calculations show that, after the cladding has been fully penetrated, component penetration could only occur several decades later. Furthermore, such penetration would result in a slow leak of primary coolant and in no way lead to what your article describes as "catastrophic consequences involving the total loss of primary coolant".

As concerns the last paragraph of the article, we have never been informed of any statements which contradict our position.

Yours faithfully,

JACQUES GAUSSENS

Framatome, Paris, France

The technical experts must be heard

SIR, — In your issue of 3 January (page 1) you had a short editorial entitled "Nuclear power: the critics must be heard". This seemed to me entirely sound but it is only half the story. Surely it is at least as important that the technical experts should be heard, and indeed listened to?

It is of course possible that the experts are biased, but it is also possible that critics are biased and while senior engineers might be unwilling to disclose all the relevant facts, you do not become a senior engineer by either ignorance of or misrepresentation of the technical facts of your profession and it is likely that the facts presented by such people are correct and worth listening to even if it is fair and reasonable for the critics to be exploring for facts which have not been released.

It must be apparent to anyone who has been watching the field that the media, especially the broadcasting media, frequently present as facts some quite wild guesses by critics and not infrequently fail to present facts given by the other side at all. A particularly serious example of this was a BBC programme towards the end of last year in which Dr Alice Stewart was given a sensible amount of time to discuss the reasons why she believes that doses of radiation are some 20 times as likely to produce cancer as is expected by the International Commission for Radiological Protection.

In a long taped interview Dr Reissland of the National Radiological Protection Board gave a number of detailed reasons for believing that Dr Stewart's statistical analysis of cancer rates at Hanford, which supported her thesis, was unsound and that the experimental data were inadequate to contradict the much larger amount of evidence taken into account by the ICRP. These criticisms have not been effectively refuted, but they were not even presented. The only statement by Dr Reissland which appeared on the programme was his answer to a question by the compère at the end of his interview asking him what would be the effect on radioactive regulations if Dr Stewart's findings were correct, to which Dr Reissland's answer was, when pressed, that *if* they *had* been correct it would involve a tightening up of regulations by a factor of 20. Without giving his previous explanation of the reasons showing that her conclusions were not supportable, this gave a complete misrepresentation for which no apology has ever been made.

Yours faithfully,

J. H. FREMLIN

Department of Physics, University of Birmingham, UK

Civil liberties and the nuclear critics

SIR, — Robin Grove White (20-27 December, page 774) has written a thoughtful and stimulating article on the alleged threat to civil liberties presented by an extended nuclear power programme. Unlike many (even most) critics of nuclear power, he is fair-minded enough to state that it is "conceivable" that alternative strategies would be worse. To his further credit, he takes seriously the possibility of "spying and infiltration of campaigning groups", even if he appears unsympathetic to the counter-measures available to the democracies.

However, I feel that it is unfair to intimate that there is anything peculiar to nuclear power about this latter aspect. The problem of uninvited involvement in our affairs seems to be with us anyway, as is indicated by such cases as those of Alger Hiss (according to a recent book), Philip Agee (according to a former CIA director) and Antony Blunt (according to himself). It is something which, in my opinion, the organisers of campaigns and demonstrations ought to face up to much more than they now do.

A problem to which Mr Grove White does not address himself at all is that of the tactics of some of the anti-nuclear people themselves. There have already been cases, in several countries, of vandalism and bombing. We can, I suppose, expect this behaviour to worsen if (as I expect and support) we choose to increase the contribution of nuclear power to the solution of our energy problems. At what point would these methods become an "impact . . . on civil liberties"? If kidnappings start? Or assassinations? This is by no means a rhetorical question. To put it another way, at what stage can we expect civilised and decent critics of nuclear power to abandon their position because of the activities of some of their associates?

Yours faithfully,

J.F. CRAWFORD

Klein Doettingen, Switzerland

Dioxin detection

SIR, — With regard to Dr Warren Crummett's comments (24 January, page 330) on my report of Dow Chemical's poor performance in detecting TCDD (dioxin) (8 March 1979, page 109), I should like to make the following points.

- Dr Crummett was asked to comment on his results at the EPA meeting and he told me that they were "poor" and "worse" than normal.
- Most of Dr Crummett's criticism of my report refers to the detection of TCDD samples below 9 parts per trillion. He is of course correct to say that the collaborators concluded that 9 ppt was the lowest acceptable detection limit and I made this point in my report.
- To my mind one of the most important issues is the reliability of measurements to detect TCDD in the 9-81 ppt range. Dow did not perform as well as the other laboratories in this study and the EPA report comparing all the results from the five laboratories bears testimony to this. Dr Crummett's comments to me at the EPA hearing only confirmed what was already in the EPA document.

Yours faithfully,

ALASTAIR HAY

University of Leeds, UK.

SIR, — My comments on Dr Hay's response are as follows:

I agree with Dr Hay that I used the words "poor" and "worse than normal", referring to the entire EPA Dioxin Implementation Plan study as "poor" and the Dow results in particular as "worse than normal".

The important issues in the reliability of the measurement of TCDD are, in order of importance: false positive results, false negative results, precision and accuracy of measurements, and confirmation by a second laboratory.

False positive results are most important because agencies in the United States, as explained by EPA's Tom Holloway at the Dioxin Implementation meeting, have a license to regulate whenever a toxic chemical is detected in environmental samples by state-of-the-art analytical methodology. Dr Hay is correct, however, in stating that the reliability of measurements in the 9-81 ppt range is scientifically important. In this range Dow and Wright State University produced comparable results on the same samples. No other laboratories examined identical extracts. So Dr Hay's comment that "Dow did not perform as well as the other laboratories" is his opinion.

I trust that this matter can now be put to rest without creating a continuous debate in your journal.

Yours faithfully,

WARREN B. CRUMMETT

Dow Chemical, Michigan, US

Suspicion of totalitarian ideologies

SIR, — Vera Rich in the article "Berufsverbote continues in spite of resolutions" (6 December, page 549) mentions the political disruption of academic life during the 1930s and remarks that it is strange that the anti-Berufsverbot campaign started so late.

But it is the 1930s that have made us suspicious of any totalitarian ideology. Left and right terror is no different, and we have already had more than enough disruption by Marxists in our universities. We should not help them to occupy influential positions in government service unless we want the suicide of democracy. There is a German proverb which says "Nur die allerdümmsten Kälber wählen ihre Schlächter selber" (only foolish calves choose their own slaughterer).

Yours faithfully,

ROLF SEIFERT

Universität Hamburg, West Germany

Was there a Maunder Minimum?

from Christopher Cullen

J.A. EDDY's claim (*Science* **192,** 1189; 1976) that solar activity was at an unusually low level during the years AD 1645–1715 (the 'Maunder Minimum') has so far seemed to be supported by the existence of a gap in East Asian naked-eye sunspot records covering this period. Two Chinese researchers at the Nanking Purple Mountain Observatory, Xu Zhentao and Jiang Yaotiao, have now drawn data from previously unused sources, and claim that on this basis solar activity can be seen to have continued unabated during the entire seventeenth century (*Nanjing Daxue Xuebao* (*Ziran Kexue Ban*) No 2, 31; 1976).

It is now acknowledged that the records of the Chinese State Astronomical Bureau form an invaluable body of data on celestial phenomena of all kinds, covering a period of two millennia. These data have come down to us in the form of digests given in the astronomical sections of the standard histories, *cheng shih*, of successive dynasties, which have in turn formed the basis of such works as Ho's catalogue of comets and novae (*Vistas in Astronomy* **5**, 127; 1962) and the special study of supernovae by Clark and Stephenson (*The Historical Supernovae*, Oxford, 1977). The first thorough listing of sunspot observations was that of Kanda (*Proc. Imp. Acad. Japan* **9**, 293; 1933), who also included Korean and Japanese data. Similar ground was covered in China recently by the Ancient Sunspot Records Research Group (*Tianwen Xuebao* **17**, 218; 1976), who in addition searched for short and long-term periodicities in solar activity over the past 2,000 years. Clark and Stephenson (*Q. Jl R. astr. Soc.* **19**, 387; 1978) have rechecked the sources used by these studies and find them to have located all relevant data. There is indeed not one sunspot recorded for the period AD 1645–1715.

Xu and Jiang point out, however, that such a gap in the records does not prove that no sunspots were observed by Chinese astronomers. In the first place, the lack of records can be partly explained by the fact that the *Ch'ing shih kao*, the draft history of the Ch'ing dynasty covering the period AD 1662–1911, simply omits to give a sunspot chronology similar to those in earlier histories. The Peking observatory was occupied by Allied troops in 1900 during the Boxer Rising, and some records may well have been destroyed. Further, apart from the standard histories compiled by central government there are a large number of local records, *fang chih*, published by local authorities or on the initiative of private persons. It is from the *fang chih* that Xu and Jiang have drawn new data. Nineteen different *fang chih* contribute 21 previously unknown naked-eye sunspot records from the seventeenth century, of which six fall into the period claimed by Eddy as the time of the Maunder Minimum. With those previously known, the total of seventeenth-century naked-eye sunspot records from East Asia now comes to 33 (see Table 1).

The six observations in the period AD 1645–1715 occur at a mean interval of 11½ years. In Kanda's catalogue the mean interval between records is 12½ years averaging over the period 28 BC–AD 1743, and Eddy himself expects to find 6 or 7 naked-eye sunspot observations in 70 years of normal solar activity as we know it today. Xu and Jiang thus conclude that solar activity in the latter half of the seventeenth century was essentially normal and claim that the Maunder Minimum is an artefact of insufficient data. After an analysis of all the seventeenth-century data they confirm that the period of variation in activity was then, as now, approximately 11 years, and propose revised dates for the maxima and minima given by Wolf and by Schove (*J. geophys. Res.* **60,** 127; 1955).

Table 1 Summary of all known seventeenth century naked eye sunspot records from Far Eastern sources.

No.	Year		No.	Year		No.	Year	
1	1603	K	12	1621	A	23	1638	K
2	1604	K	13	1624	K	24	1639	K
3	1613	F	14	1624	K	25	1639	K
4	1616	K	15	1624	K	26	1639	F
5	1616	F	16	1625	F	27	1643	F
6	1617	F	17	1626	F	28	1647	F
7	1617	K	18	1631	F	29	1650	F
8	1618	F	19	1635	F	30	1655	F
9	1618	F	20	1637	F	31	1656	F
10	1618	K	21	1638	F	32	1665	F
11	1618	F	22	1638	F	33	1684	F

A, Taken from Ancient Sunspot Records Research Group list.
F, *fang chih* record from Xu and Jiang.
K, Kanda.
Full texts of original observations are given in Xu & Jiang.

Statistical analysis of the *fang chih* data on sunspots and other transient celestial phenomena must be approached with caution. In the case of the *cheng shih* standard histories we have a series of data covering successive periods of operation of the State Astronomical Bureau. We may begin with the assumption that the Bureau did its observational work with uniform efficiency and that the historians digested their data with equal thoroughness in each dynasty. The next step is to attempt to correct for the sampling error introduced by periods of political chaos or of simple incompetence in the running of the Bureau; the completeness of records of other types of phenomenon provides a useful cross-check here. The results of this process may still be open to doubt, but we can at least be sure that at any given time we are only dealing with one group of observers, and that their data are being transmitted to us through a single channel. In the case of the *fang chih*, however, the situation is much more complex. We have little or no idea who made the observations they contain or how the data came to be recorded; this is a source of randomness absent from the central government records. Further, it is quite unsafe to assume that the *fang chih* taken together present an unbiased sample of data. In comparing the frequency with which sunspots are recorded in different periods, we should remember that some periods will be more intensively covered by *fang chih* records than others. Before the required weighting can be applied to Xu and Jiang's data a considerable amount of

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Christopher Cullen is a Research Fellow of Clare Hall, Cambridge at present working at the Kyoto University Research Institute for Humanistic Studies, Japan.

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bibliographical research will be needed.

The dangers of sole reliance on the official records are now clear. For example, Clark and Stephenson have suggested that a phenomenon similar to the proposed Maunder Minimum may have occurred under the Ming dynasty between AD 1400 and 1600. They argue that since contemporary official records of other astronomical phenomena are relatively complete, the gap in the Ming standard history sunspot records for that period must be regarded as significant. However, as we have seen, an identical argument applied to the Ch'ing standard history would lead to the patently false conclusion that there were no sunspots at all between AD 1662 and 1911. Given the civil and military chaos at the end of the dynasty, a section of the Ming records may simply have gone missing in the same way as under the Ch'ing. The work of Xu and Jiang has shown that such points can only be decided after a search of the literature ranging outside the confines of the official sources. As well as the large number of local histories, such a search must attempt to cover the essays and common-place books, *pi chi*, of private scholars likely to have had astronomical interests; the yield from these will be considerably smaller, but still worth the effort. Until the results of such an arduous undertaking become available, judgment must be suspended. □

The 'blueprint hypothesis' of axon guidance

from David I. Gottlieb

DURING the development of the vertebrate central nervous system axons grow over long distances and bypass vast numbers of neurones to home in on a distinct target region where they form synapses with a characteristic subset of neurones. Although it has long been appreciated that axons have some means of recognising their final destination (see *News & Views* **282**, 672; 1979) it needs to be emphasised that axons also take a sterotyped route to their destination, they do not, for example, branch extensively and resorb the collaterals which fail to hit the target. How are these routes through the developing brain marked? Some recent anatomical investigations by M. Singer and his colleagues suggest that certain non-neuronal cells of the regenerating and developing central nervous system may have a crucial role.

Evidence for this view first emerged from anatomical studies of the regenerating lizard spinal cord. Egar *et al.* (*J. Morphol.* **131**, 131; 1970) showed that during the early stages of regeneration the cord consists of an epithelial tube formed predominantly of a single layer of cells called ependymal cells surrounding a central canal in continuity with the original spinal canal. An unexpected and exciting finding was that the distal portions of the ependymal cells (that is, the surface away from the lumen of the central canal) were arranged to form large extracellular channels which contained only an electron lucent amorphous material. During subsequent stages of regeneration these channels became choked with axons growing along the length of the cord and presumably originating from neurones located more rostrally. Similar ependymal channels were found in the regenerating cord of the newt (Egar & Singer *Expl Neurol.* **37**, 422; 1972).

Numerous anatomical investigations of the central nervous system during early development have revealed the presence of large, cell free spaces but these have usually been written off as fixation artefacts. However, in studies of the embryonic newt cord Singer *et al.* (*J. comp. Neurol.* **185**, 1; 1979) found large channels between ependymal cells that bore a striking similarity to those of the regenerating cord. As in the regenerating cord, the empty channels became filled with growing axons as development proceeded. In both regeneration and development the anatomical impression was clear: ependymal channels seemed to guide bundles of growing axons. Singer *et al.* proposed what they call the 'blueprint hypothesis', the central tenet of which is that ependymal channels provide the important cues which guide developing axons and result in the characteristic route taken by the major pathways of the brain. Along similar lines Katz and Lasek (*J. comp. Neurol.* **183**, 817; 1979) have performed experiments in which eyes were grafted onto the trunk of developing frog embryos; axons from these eyes enter the spinal cord and grow for long distances in a highly circumscribed tract near the lateral margin of the cord. Some substrate clues must channel these aberrant axons and ependymal channels seem like reasonable candidates. Evidence for cellular channels has also been found in the embryonic mouse retina (Silver & Sidman *J. comp. Neurol.* **189**, 101; 1980). In this case the channels appear transiently during development and seem to be situated in a way that would guide ganglion cell axons back into the optic stalk as they grow. Doubtless such cellular channels will be found in other regions of the embryonic central nervous system.

Do the channels actually guide growing axons in a way which can explain the characteristic paths of the major tracts in the central nervous system? One problem with this idea is that developing tracts in the embryo are relatively long (from several to tens of millimetres) while we don't really know how long the channels are. Serial reconstruction studies are needed to settle this point. Another is that a clean test of the idea such as altering the channels to see what happens to the tracts seems impracticable. Thus it may be a long time before we know whether ependymal cells carry some of the elusive molecular markers for specificity. Whatever the final outcome, ependymal cells and channels will be objects of lively experimental interest as neurobiologists try to understand how tracts find their way. □

David I. Gottlieb is Associate Professor of Neurobiology, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis.

How plants exploit animals

from Peter D. Moore

THE ecological position of most green plants, at the base of food chains, has placed them in what seems to be a rather vulnerable position. It is natural that many of their adaptations during the course of evolution have been defensive; both morphological and biochemical features have frequently been modified so that the grazer should regard them with more respect than relish. Notable exceptions exist, of course, such as the use of animals as vectors in the transport of seed and pollen. Here the plant is at pains to excite an animal's greed in order to exploit its freedom of movement, thus rendering more effective the dispersal of the species and the free exchange of genes. The ecological and evolutionary rewards of these processes have evidently justified the necessary energetic investment.

In view of the advantages gained by plants in these mutualistic associations with their mobile dependents, it would be surprising if their response to direct grazing were entirely passive. In a systems analytical approach to this question, Lee and Inman (*Ecology* **56**, 1455; 1975) concluded that although grazers consume only a very small proportion of primary production (see *News & Views* **282**, 443; 1979), they exert a dampening effect on the fluctuations in plant production which result from environmental oscillations. In this way they may contribute to the stability

Peter D. Moore is a Senior Lecturer in the Department of Plant Sciences, King's College, London.

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of the ecosystem, which could be considered a benefit to all member organisms.

Owen and Wiegert (*Oikos* **27**, 488; 1976) sought for more tangible benefits to plants from the existence of a contingent of grazers in their midst. They asked the question in the forcible form "Do consumers maximize plant fitness?", and their guarded but affirmative reply was based on some detailed illustrations from nature. For example, they use the figures of Llewellyn (*J. appl. Ecol.* **9**, 261; 1972) to show that a population of a million aphids inhabiting a single lime (*Tilia*) tree, 14 m in height, could release sugar as honeydew at the rate of 407 g day^{-1}. Llewellyn's overall annual estimate of sugar rain was 1 kg m^{-2}. This will obviously have a profound influence on soil microbial activity and Owen and Wiegert single out for attention the free-living nitrogen-fixing microbial genus *Azotobacter*. The energetic stimulation of this component of the soil flora could result in higher rates of nitrogen input to the soil and consequently the ultimate encouragement of growth in the vegetation. This assumes, of course, that *Azotobacter* will be the sole or main beneficiary from the aphidic manna, which is not necessarily so, since the ecology of the competitive interactions among soil microbes is likely to be complex, but it is nevertheless reasonable to suppose that some increase in nitrogen fixation will occur. The important question is whether the nitrogen gain to the plant is worth the carbon loss.

A still more direct example of a stimulating consumer has been provided by Boscher (*Oikos* **33**, 451; 1979) in her studies of the cultivated leek, *Allium porrum*. This plant is frequently blighted by the leek moth *Acrolepiopsis assectella*, the larvae of which feed on leaves, scapes and pedicels, though not normally on the flowers, which Arnault (*C. hebd. Acad. Sci Paris* **280**, 2487; 1975) considered to be toxic to them. Boscher found that moderate defoliation of the leek had no adverse effect on fruit production, indeed some plants produced more fruit. Severe defoliation resulted in a considerable development of vegetative bulbils in the inflorescence. This is a reproductive strategy which allows rapid expansion of the population, since the bulbils produce plants which are sexually mature in their first year whereas plants grown from seed only flower in their second year. The switch to vegetative propagation is thus controlled by the consumer organism.

There are, of course, some plants which have themselves turned predator; some fungi trap and consume detritivorous arthropods, and there are the insectivorous pitcher-plants and sundews. But a more subtle system for the exploitation of animals as an energy resource has been developed by some tropical epiphytes which have formed associations with ants. These were first described back in the eighteenth century, but have recently been examined more closely by Janzen (*Biotropica* **6**, 237; 1974) and Huxley (*New Phytol.* **80**, 231; 1979). The stems of these plants are swollen and become hollow as a result of tissue death early in the life of the plant. The inner surfaces of these cavities are covered with warty protrusions. The hollow stems are invariably occupied by ants, and often by a variety of other organisms. The ants deposit quantities of detritus within these cavities and it was suggested during the last century that this could provide a source of inorganic elements for the plants if they were able to absorb them.

In laboratory and field experiments on two of these ant plant species, *Myrmecodia* and *Hydnophytum*, Huxley found that, in the presence of ants or of added inorganic nutrients, the plants grew more rapidly than without these. When ants were fed on honey water containing ^{32}P as phosphate and ^{35}S as sulphate and as methionine, subsequent autoradiography showed an accumulation of radioactivity around the surfaces of the warts and also in the plant vascular tissue. So the plant can absorb these elements, possibly by way of faecal material in the cavities. Rickson (*Amer. J. Bot.* **66**, 87; 1979) has conducted some similar experiments in which he provided the ant plant and its resident ants with a supply of fly larvae labelled with ^{14}C. Within 6 to 8 weeks radioactivity could be detected throughout the plants, demonstrating absorption and translocation of organic material either directly from the decaying fly larvae or indirectly through ant faeces.

Rickson proposes that the ants donate a proportion of their food to the host plant,

The parity of the pion

from John Edgington

TEXTBOOKS of nuclear physics are not noted for their flair and style. A rapidly developing subject, using difficult theoretical techniques to interpret and codify great quantities of experimental data, nuclear physics rarely affords us glimpses of the essential simplicity of nature, and the standard pedagogy tends to reflect this. However a result recently obtained by C. Wilkin (*J. Phys. G*, L5, 1980), though not new, is so refreshingly simple in its derivation that it should rapidly find its place in undergraduate courses. The topic is the intrinsic parity of the π-meson.

The original (and so far only) determination of the pion's parity was that made by Panofsky at Berkeley in 1951, confirmed in 1954 by Steinberger at Columbia. Drawing on an idea of Ferretti dating from 1946, the argument has been outlined in many textbooks since, notably in Bethe and the Hoffmann's semi-classic 'Mesons and Fields'. The reaction studied is $\pi^- + d \rightarrow 2n$; arguing from general conservation principles one shows that the very existence of this reaction requires the pion to have negative parity. The reasoning is unambiguous but requires knowledge of the initial quantum state of the π^-d system; the argument is that the pion cascades down through its electron-like orbits until the final strong interaction occurs in the lowest ($l = 0$) orbit. This assumption, though highly plausible, is not absolutely compelling. That the pion's parity is negative is not seriously in doubt, but it would be comforting to have a slightly more watertight experimental proof.

Wilkin has provided such a proof. He uses some results obtained at SIN, Switzerland, by a group led by R. Hess of Geneva University, and reported to the 8th ICOHEPANS* Conference at Vancouver last August. Hess *et al.* have been studying the reaction $p + p \rightarrow \pi^+ + d$, using a polarised beam and target; they have measured the dependence of the cross section on the relative spin orientations of target and projectile. Their motivation was to provide further data on the spin-dependence of the nucleon–nucleon interaction, and the present result is an amusing and unexpected bonus.

Wilkin points out that, in the forward direction, there is a simple relation between two coefficients, A_{LL} and A_{TT}, both of which measure the difference in reaction probability between states whose proton spins are aligned parallel, and anti-parallel, to one another. A_{LL} refers to the case where the spins are directed parallel (or anti-parallel) to the projectile's motion, while A_{TT} refers to the case where the spins are at right angles to this. Wilkin shows that

$$A_{LL} \pm 2A_{TT} = 1$$

the positive (negative) sign being taken if the pion has positive (negative) parity. By making a small extrapolation of the data of Hess *et al.* to the forward direction, he finds $A_{LL} = -0.3$, $A_{TT} = -0.6$; these values, with their rather small uncertainties, conform very comfortably with the notion that the parity of the pion is negative. Positive parity is unambiguously ruled out. Since A_{LL} may not exceed unity, positive parity would forbid A_{TT} from being negative, but it manifestly is so. This simple, yet pleasing demonstration of the pseudoscalar nature of the pion seems to have no controversial aspects.

John Edgington is a lecturer in the Department of Physics, Queen Mary College, London.

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but this implies both that the ants carry in material in excess of the ant colony's needs and that the route of movement of ^{14}C is directly from the food material to the plant. From the data available at present, it is still possible that the ants are acting entirely out of self-interest and are consuming all food entering the cavity so that ^{14}C entering the plant is derived only from faeces. If this latter explanation is correct, then the ant loses nothing from the association since there is no undue energy investment on its part. In either event, the plant very obviously gains from the association in both its organic and inorganic uptake. The presence of the ants must therefore represent an energy resource for the plant which supplements its photosynthetic intake. It could be regarded as a symbiotically adapted facultative saprophyte.

The coevolution of plants and animals has led to such very obvious structures as flowers and thorns and to the evolution of a variety of chemical compounds from tannins to cardiac glycosides. It should not come as any surprise, therefore, to find that in taking advantage of the grazer the apparently passive plant is less green than it's cabbage-looking. ☐

New ways of applying pesticides

from Graham Richards

AT the biochemical level there is much in common between medical pharmaceutical research and the development of pesticides for agriculture. The great difference lies in overcoming the problem of application which is so much more difficult when dealing with plants. Researchers in this area have likened their problem to attempting to cure the common cold by dropping aspirins from an aircraft and hoping to catch a fair percentage of the population looking skywards with their mouths open. Small wonder then that the paper which caused the greatest interest at the 1979 British Crop Protection Conference held in Brighton* was the description by R.A. Coffee (ICI Plant Protection Division, Haslemere, Surrey) of an electrodynamic method suitable for the deposition of ultra-low volume droplets of insecticides, fungicides and herbicides.

In principle, the technique is akin to the famous Millikan oil drop experiment whereby the charge of the electron was first measured, and is similar in some respects to methods used in paint spraying. The sensational qualities are that Coffee's new design is free of any moving parts and can be run on torch batteries. Above all the droplets have curved trajectories so that when spraying the front of a plant the chemical is also delivered to the back.

The system which can be hand-held uses a gravity feed to supply liquid to a high-voltage nozzle. This voltage is achieved from the torch batteries using a solid-state converter. The emergent charged liquid drops are subjected to an intensely divergent electric field which is sufficient to carry the droplets to the target which is at earth potential. This field strength is about two orders of magnitudes greater than the gravitational pull, making for very much reduced drift and wind influence.

The almost magical quality of being able to deliver pesticides to the hidden surfaces of a plant when apparently spraying only one side arises from the curved flux lines of the electrically propelled droplets. As the Poisson equation demands, the lines of force are orthogonal to the equipotential contours so that the spray diverges from the high voltage nozzle only to converge on the earthed target and thus to penetrate to surfaces which are not visible on a direct line of sight from the sprayer.

A nozzle flow rate of about 0.1 ml s^{-1} corresponds to a spray volume of as low as half a litre per hectare; the droplets being of about 100 μm diameter (a power of 18 mW). This ultra-low volume application has many attractions. Most obviously it should lead to a significant reduction of environmental problems associated with crop spraying. The simplicity of the device should also mean that it can be used by peasant farmers in the less sophisticated parts of the world where the introduction of chemical aids can produce as much as a 10-fold increase in land cultivation.

Finally the device is an encouragement to chemists and researchers in crop protection since if the delivery of their compounds becomes more efficient then more expensive chemicals become economically viable and one constraint on their research is at least slackened. ☐

Graham Richards is a lecturer in the Physical Chemistry Laboratory, University of Oxford.

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*British Crop Protection Conference — Pests and Diseases was held in Brighton, UK, on 19-22 November, 1979. Vol III of the Proceedings will be published in March 1980.

Inbreeding among zoo animals

from Robert M. May

IMPROBABLY located off Wiltshire Boulevard in Los Angeles is a small park containing the Rancho la Brea tar pits. Here the bones of creatures — dire wolves, sabretooth tigers, mammoths, ground sloths — from the romantically envisioned 'Rancho la Brean fauna' may be seen, both in existential clutters of bones in the original tar pits, and in analytic exhibits. These large mammals flourished in North America until comparatively recently, when 35 of 49 genera became extinct in the short period from roughly 11,000 to around 8,000 years ago; many people believe the extinctions were caused by the arrival in North America of the comparatively advanced Clovis and Folsom hunters at that time (Martin *Nature* **212**, 339; 1966).

The largest bird of those times was the now-extinct *Teratornis incredibilis,* whose wingspread of about 16 feet makes it the biggest soaring bird of prey yet to have lived. Its smaller congener, *T. merriami,* with a 12-foot wingspan and an estimated weight of 50 lbs, followed it into oblivion. The greatest bird of the Rancho la Brean fauna that survives is the California condor, with a wingspan of over 9 feet and a weight around 23 lbs; here I resolve the taxonomic pedantry of whether the Pleistocene *Gymnogyps amplus* and the present-day *G. californianus* are really two species, or the same species separated in time, by referring to both as the California condor. (Note that the three birds, which occurred together over part of their pristine ranges, form a nice Hutchinsonian sequence, with length ratios around 1.3 and weight ratios around 2; for an irreverent discussion of these ratios, see *Nature* **270**, 660; 1977.)

The California condor's chances of continued existence are, however, slim. Its Pleistocene range, west of the Rocky Mountains from British Columbia to Baja California and east of the Rockies in a great southern strip extending to Florida, has narrowed to two counties in southwestern California, in the dry, mountainous parts of the southern Coast range and the Sierra Nevada. The total population has fallen from an estimated 60 in the 1940s, to 42 in 1963, to 30 today. Some of the loss is demonstrably due to wanton shooting by idiots exercising their 'right to bear arms'. The breeding rate (typically one egg per year) is not calculated to rebound from such vicissitudes. Even this potential recruitment is not being realised, possibly because of the many disturbances associated with increasing human encroachment in the habitat, and possibly because pesticides in the food supply are weakening the birds' egg shells.

There is currently much controversy about what to do (see *NY Times*, 23 Dec. 1979, A17). Various Federal and California agencies, along with the National Audubon Society, have signed an

Robert M. May is Class of 1877 Professor of Zoology at Princeton University.

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agreement to commit resources to a "condor recovery plan". The proposal is to mark and fix radio transmitters to the surviving condors, hoping to gain more precise information about their autecology and about the causes of the continuing decline in population. The plan contemplates capturing 9 of the remaining 30 birds for captive breeding in zoos. Some conservation groups have attacked these proposals, on the grounds that (in the words of David Brower, founder of Friends of the Earth): "The condor deserves better than to be pushed toward extinction by enthusiasts of technological fixes. The proposed plan threatens to destroy the species in a misguided effort to save it". The observed trends suggest to me there is little hope for the California condor in the wild, no matter what is done.

If this gloomy view is correct, the condor will, at best, join an increasing band of creatures who live on only in zoos. There are many consequent problems. One class of difficulty has to do with getting the animals to breed in captivity; thus, although Andean condors have produced offspring in zoos, California condors have never yet done so. Another concern, which has received less discussion, has to do with the necessarily reduced genetic diversity associated with the small founding stocks of zoo populations. In particular, many zoo stocks will be inbred to a significant degree.

An important recent study of the effects of inbreeding in zoo populations is by Ralls, Brugger and Ballou (*Science* 206, 1101; 1979). They examine records bearing on the relation between inbreeding and juvenile mortality for 16 species of captive ungulates. The offspring were separated into two classes: "noninbred" for all young with unrelated parents; and "inbred" for all young whose parents were known to have an inbreeding coefficient greater than zero. The authors believe this coarse and somewhat unsatisfactory classification scheme is forced on them by the smallness of the sample size (the scheme includes the assumption that the founding individuals were not related, which is not always true). Of the inbred young for which explicit calculation of the inbreeding coefficient is possible, 75% had inbreeding coefficients in excess of 0.25. Within these two classes, young that survived 6 months or more were considered to have "lived", while the "died" category included all offspring surviving less than 6 months (including stillborn and those born prematurely).

The results are shown in the Table. The intuitive impression of a significantly higher juvenile mortality associated with inbreeding is confirmed at the 95% confidence level for 9 of the 16 species, and the sign of the effect is in the right direction for 15 of the 16 (kudu being the exception). In a different analysis, the authors showed that for "19 of 25 individual females, belonging to ten species, a larger percentage of young died when the female was mated to a related male than when she was mated to an unrelated male".

Juvenile mortality of inbred and noninbred young.

Species	Noninbred young		Inbred young	
	Lived	Died	Lived	Died
Indian elephant	11	2	2	4
Zebra	20	7	3	2
Pygmy hippopotamus	139	45	23	28
Muntjac	18	4	12	6
Eld's deer	13	4	0	7
Père David's deer	15	2	19	3
Reindeer	19	10	9	12
Giraffe	11	3	2	3
Kudu	10	4	8	3
Sitatunga	15	1	31	28
Sable	18	4	3	7
Scimitar-horned oryx	35	2	0	5
Wildebeest	6	1	29	12
Dik-dik	10	7	7	8
Dorcas gazelle	36	14	17	25
Japanese serow	52	21	27	35

This work is controversial. Although the deleterious effects of inbreeding are well known in animal husbandry, many conservationists and wildlife managers have argued that "Adverse comment about the ill-effects of inbreeding is . . . often exaggerated, and provided the area is well looked after and not overstocked, there should be no harmful effects" (Whitehead, in *Threatened Deer,* IUCN Publ., Switzerland, 1978). I think Ralls, Brugger and Ballou make it clear this is wishful thinking, and "that deleterious effects of inbreeding are widespread in captive populations of exotic animals and have remained unrecognized only because the majority of zoos have not maintained detailed records".

It must be emphasised that nobody is suggesting that the difficulties caused in inbreeding are a reason to give up attempting to breed animals and preserve species in zoos. It is simply that these difficulties are real, and that they need to be addressed constructively (by genetic management of small populations whenever practical), rather than ignored.

Nonlinear dynamics

from N. MacDonald

NONLINEAR dynamics is an explosively expanding subject, and a recent meeting* brought together more than 400 people from a great range of disciplines, from fluid dynamics to biomathematics, with a common interest in the recent advances in understanding nonlinear phenomena.

A major part of the meeting was devoted to studies, both experimental and theoretical, of the onset of turbulence. Turbulence is characterised by complex structures, on many different scales, in both space and time. However, the onset of turbulence, that is to say the first appearance of modes of motion more complex than laminar flow, is usually studied in the time domain, by analysis of the frequency spectrum of an appropriate experimental quantity. Traditionally, the transition from simple to complex frequency spectra characterising the onset of turbulence has been thought of as analogous to a series of bifurcations of the trajectories of a dynamical system (a system defined by a finite set of ordinary differential equations). The first transition is from a steady state to a closed curve with a definite frequency of transit; the second from that closed curve to motion on a torus (quasi-periodic motion with two incommensurable frequencies), and so on. More recently the possibility has been recognised that after one or two such bifurcations there may be a sudden jump to aperiodic motion. In the experimental data presented at this meeting (for example by G. Ahlers, Bell Laboratories, Murray Hill, and J. Gollub, Haverford College) the following features have been identified:

(1) Appearance of new incommensurable frequencies f_1, f_2 . .

(2) Entrainment of these frequencies so that (for example) f_1, f_2 approach values f_0, $2f_0$.

(3) Sudden appearance of aperiodic spectra.

(4) Appearance of subharmonics $f_1/2$, $f_1/4$, . . successively.

Certain of these features were also present in the data described by C. Vidal (CNRS, Talence) on the temporal behaviour of a stirred Belousov-Zhabotinsky chemical reaction, as the flow rate through the reaction vessel is increased.

Some reservations must be expressed with regard to this way of thinking about turbulence. As emphasised by P.C. Martin (Harvard University) essential aspects of the physics may be missed if one does not consider the spatial aspects of turbulence.

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*A conference on 'Nonlinear dynamics' sponsored by the New York Academy of Sciences and organised by Dr. R.H.G. Helleman, Twente University of Technology, was held in New York on 17-21 December, 1979.

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Further, the appropriate theoretical framework for fluid dynamics, the Navier–Stokes equation, is defined on an infinite-dimensional phase space, that of the velocity vector field. D. Ruelle (IHES, Bures-sur-Yvette) reviewed the progress that has been made in understanding how far it is safe to apply the concepts of dynamical systems to solutions of the Navier–Stokes equations.

Within dynamical systems there are two important dichotomies; on the one hand that between dissipative systems — the category to which any presumed analogue of fluid dynamics must belong — and Hamiltonian systems, and on the other, within Hamiltonian systems, between the integrable and non-integrable cases. J. Moser (Courant Institute, New York) reviewed recent progress in understanding integrable systems. The most difficult of the long-established integrable cases, were solved by the method of separation of variables in the Hamiltonian-Jacobi equation. The recent zoo of curious integrable cases, such as the Toda lattice, has been studied in terms of the spectral theory of linear operators. It is now possible to trace a mathematical relationship between these two apparently diverse methods, and to give a systematic discussion of the connection between the various special cases.

The classical theory of integrable systems played a considerable part in the early development of quantum theory. As early as 1917, while the Bohr–Sommerfeld semi-classical method was being actively pursued, Einstein pointed out that this method could not hope to handle any non-integrable classical problem. The rise of quantum mechanics diverted attention from this, but in recent years the practical usefulness of semi-classical methods in atomic and molecular physics has led some authors to return to this problem. M.V. Berry (University of Bristol) reviewed the work which he and his associates have carried out on the quantisation of some well-known non-integrable systems, such as Arnold's cat and Sinai's billiards (see for example M.V. Berry *et al. Ann.Phys.* **122,** 26; 1979).

The trajectories of integrable Hamiltonian systems stay on energy surfaces of dimension lower than that of the phase space. Some or all of the trajectories of non-integrable systems fill up (in an imprecise sense) regions of the phase space (ergodic behaviour). There has been some controversy regarding the universal nature of a mechanism (Arnold diffusion) proposed for the transition from quasi-periodic to ergodic behaviour of the trajectories of a perturbed integrable Hamiltonian system. A numerical experiment bearing on this was reported by M.A. Lieberman (University of California, Berkeley and Imperial College, London). A particle is bounced between a plane wall and a parallel rippled wall. A transition can occur between trajectories confined to a single dimple and trajectories wandering over the plane.

Another aspect of the appearance of seemingly random features in a deterministic physical model was the subject of a talk by J. Ford (Georgia Institute of Technology, Atlanta). The model is a set of particles on a line, which are alternately free and harmonically bound. For low enough frequency of the bound particle oscillations, any blow on the end particle is transmitted as a wave pulse along the line, distance travelled being proportional to time elapsed. As the frequency is raised to a point at which there is a significant incidence of multiple collisions (a free particle striking its right-hand neighbour more than once before next striking its left-hand neighbour) the nature of the transmission of information changes. The wave pulse is damped out and the disturbance propagates like heat instead of sound, with the distance travelled proportionate to the square root of the time.

Just as the Navier–Stokes equation can be illuminated by cautious extrapolation of results from dynamical system theory, so the study of complex behaviour in dynamical systems received a considerable impulse from the striking properties of discrete mappings. The degree of complexity attained by the solutions of such apparently trivial recurrence relations as

$$\alpha_{m+1} = \alpha_m(1-\alpha_m)$$

is now familiar (see the review by May, *Nature* **261,** 459; 1976). Much attention was paid at the New York meeting (for example in the talks by O. Lanford, Berkeley, E.N. Lorenz, MIT and M.J. Feigenbaum, Los Alamos) to the remarkably precise scaling laws that apply to these solutions. In general, for the mapping

$$x_{m+1} = f(a, x_m)$$

where for fixed a the function f has a single maximum in x, there are, as a passes through values a_n, successive bifurcations from a periodic solution of order n to a periodic solution of order $n+1$. Feigenbaum has found that the limit for large n of the ratio

$$\frac{a_n - a_{n-1}}{a_{n+1} - a_n}$$

has the value 4.6642016 . . He conjectures (for details see *Phys. Lett.* **A74,** 375; 1979) that similar laws may hold for the subharmonic bifurcations observed in the experiments on the onset of turbulence.

This was undoubtedly a timely and stimulating meeting. The predominant impression was of a fruitful interplay between the construction of special models illustrating striking aspects of nonlinear dynamics, and the exploration of the underlying mathematical structures. □

N. MacDonald is in the Department of Natural Philosophy, University of Glasgow.

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100 years ago

DIFFUSION OF COPPER IN THE ANIMAL KINGDOM

The fact of the normal presence of minute quantities of copper in various members of the animal kingdom has been noticed by several chemists within the past twenty-five years. Kingzett states that he has invariably found it to be a constituent of the human brain, while Odling and Dupré, and Bergeron and Hoté have determined analytically the average amount of copper present in the liver and kidneys of human beings and domestic animals. In the latter case the average percentage of copper found was about 35 millionths. Some two years since Cloez examined the blood of a deer, and found it to contain 6 millionths of copper. The most interesting instance of the occurrence of copper in the animal creation is, however, that communicated by Prof. Church to the Royal Society in 1869. At this time he was engaged in the investigation of a peculiar, soluble, red colouring matter present in the wings of the Turaco, a bird from the West Coast of Africa. A thorough study of this pigment showed it to contain 5.8 per cent of copper, and Prof. Church established for it the formula $C_{50}H_{56}O_{19}N$ Cu. Led to seek the source of this strange factor in the animal economy of the Turaco, he succeeded in detecting copper in the fruit of the *Musa sapientum,* which forms the chief article of the bird's diet.

To these few isolated cases of the normal assimilation of copper in the animal Kingdom, Dr. M. Giunti, in the last fascicule of the *Gazetta Chimica Italiana* (vol. ix. p.541), adds a number of interesting and diversified instances.

His attention was first directed to the subject accidentally by finding over one-third of 1 per cent of copper in the guano deposits from bats occurring in certain Italian caves. This led to an analytical examination of the bat, the results of which showed that about four ten-thousandths of the weight of the ashes of this animal consist of cupric oxide. Still bent upon finding a more ultimate source for the metal, Giunti has subjected to analysis quantities of the insects which form the food of the bat, and in all cases he has found copper present in greater or less amount.

SIZING AND MILDEW IN COTTON GOODS

Sizing and Mildew in Cotton Goods. By G.E. Davis, C. Dreyfus, and P. Holland. (Manchester: Palmer and Howe).

The application of a certain kind of science to a certain kind of commerce is rapidly producing a literature of its own. It is not long since that we had occasion to notice a work which treated of the manner in which silks could be "weighted" by chemical means, and the volume now before us is the second of its kind which is concerned with the relations of chemistry and mycology to the manufacture of cotton goods.

It is hardly worth while to take up valuable space by noticing the merits or demerits of a book such as this, the object, or at least the tendency, of which is to show the manufacturer how, by the application of certain scientific facts and principles, he may seek to perpetuate a system which, we honestly think, is simply a gigantic fraud.

From *Nature* **21,** 29 Jan., 305, 298; 1880.

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REVIEW ARTICLE

The mechanism of protein secretion across membranes

Bernard D. Davis & Phang-C. Tai

Bacterial Physiology Unit, Harvard Medical School, Boston, Massachusetts 02115

Many secreted proteins are synthesised as a large precursor with an additional hydrophobic N-terminal signal sequence that is cleaved by a membrane-bound enzyme. The proteins are secreted as nascent chains. The work leading to the current models of protein secretion is reviewed and the value of bacterial systems in the study of protein transfer across membranes is stressed.

MOST cells, and perhaps all, secrete proteins across membranes—either directly into the extracellular fluid, or into vesicles or organelles. Study of the mechanism began with Palade's discovery, 25 years ago, that animal cells contain ribosomes bound to the membrane of the endoplasmic reticulum, as well as ribosomes free in the cytoplasm[1]. In the next few years various secretory proteins were shown to be synthesised on the bound ribosomes, and various cytoplasmic proteins on the unbound ribosomes (reviewed in refs 2, 3). This distribution suggested that secreted proteins might cross the membrane as growing chains, rather than after completion. In support of this model, Redman and Sabatini[4] used puromycin to induce release of incomplete chains from the ribosomes of microsomes (vesicular fragments of endoplasmic reticulum, spontaneously formed on cell lysis): these chains could then be released by a low concentration of detergent, which suggested that they had been transferred into the lumen of the vesicles.

To explain how the membrane-bound and the unbound ribosomes differed in their selection of messenger RNA, as well as in their location, a difference in structure was proposed. However, no such difference could be detected. It was therefore necessary to consider the alternative possibility that the ribosomes are all alike and bind mRNA randomly, and it is the mRNA that determines their location (as well as their product). In 1971 Blobel and Sabatini[5] proposed that the mRNA might play this role indirectly, by coding for a special sequence near the beginning of the polypeptide chain that might be recognised by a membrane binding factor; the chain would then presumably be secreted while still growing.

Such *N*-terminal signal sequences were subsequently demonstrated on many secretory proteins. Nevertheless, that did not establish whether binding of the signal is followed by cotranslational secretion or whether the chain is first completed, then folds against the membrane surface and is subsequently transferred by engulfment by the membrane after release from the ribosome. In fact, as we shall see, both mechanisms can be found.

Recently cotranslational secretion has been directly demonstrated, particularly through the study of protein secretion in bacteria. Moreover the use of bacterial systems has allowed new approaches to the study of secretion. These approaches are based on the accessibility of the relevant bacterial membrane surface (in contrast to the endoplasmic reticulum of eukaryotic cells), and the isolation of bacterial mutants altered in secretion. In this review we shall emphasise these studies, briefly noting closely related work on eukaryotic cells, and on the formation of membrane proteins.

The signal sequence

In addition to its affinity for membrane, the postulated special signal sequence turned out to have the unpredicted property of being cleaved from a precursor protein during secretion. On this basis the existence of such a sequence was discovered adventitiously, and independently of the suggestion of Blobel and Sabatini[5], by Milstein and coworkers[6] in 1972. Specifically, during a study of *in vitro* translation of mRNA (from myeloma cells) into an immunoglobulin, these workers observed that in the absence of homologous membrane the immunoglobulin light chain was formed not as such but as a larger precursor; moreover, the extra sequence, of MW ~ 3,000, was *N*-terminal. Milstein *et al.* therefore proposed that the extra sequence provided a signal for attaching the growing chain of this secretory protein to the membrane. This group further reported[7] that during protein synthesis homologous membrane fragments can convert the precursor to the ultimate product; but after completion of the precursor the cleavage site evidently becomes inaccessible to the enzyme. Other laboratories also noted the synthesis *in vitro* of a larger precursor of an immunoglobulin chain[8-11].

These findings provided the first evidence that proteins destined for secretion have a special signal sequence, and that this sequence is removed after it has served its function of initiating transfer. Moreover, Schechter and coworkers[10,12,13] showed that the signal sequence of an immunoglobulin light chain precursor has a marked preponderance of hydrophobic amino acids—a property that would fit the proposed attachment of this sequence to the membrane.

Subsequently a series of more refined studies (reviewed in ref. 14) was undertaken by Blobel and coworkers[15-17], culminating in the determination of the complete amino acid sequence of the signal segment of a number of precursor proteins from eukaryotic cells. In some cases the membrane enzyme(s) that cleave this sequence were also demonstrated. These studies led to increased interest in the 'signal hypothesis', and to a more detailed model in which the signal sequence induces aggregation of membrane proteins to form a tunnel around the secreted chain[15].

More recently, precursors with an *N*-terminal signal sequence have also been demonstrated[18-20], or inferred from the corresponding DNA sequence[21], for several proteins that are secreted from bacteria. In addition, both in bacteria[22-26] and in animal cells[27], precursors with cleavable signal sequences have been demonstrated for several proteins destined for incorporation into the membrane, rather than for secretion. The process of incorporation has therefore been viewed as a model for secretion (see, for example refs 26, 28). This view is supported by evidence (see below) that the two kinds of protein compete for the same sites of entry (even though their interaction with the membrane later becomes quite different).

The signal segments for various proteins have been found to vary considerably in sequence, and even in length. Representative examples are presented in Table 1; a more complete tabulation can be found in ref. 28.

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Direct evidence for cotranslational secretion

Cotranslational secretion of a protein would be demonstrated directly if one could label the end of the growing chain protruding from a membrane and show that the other end was still attached to a ribosome as peptidyl-tRNA. Such experiments seemed feasible in bacteria (although not in eukaryotic cells), since the far side of the bacterial plasma membrane should be accessible to external modification (Fig. 1).

Efforts to label protruding chains were therefore undertaken in our laboratory[32]. In growing cells of *Escherichia coli* the polysomes were stabilised by rapid chilling plus addition of chloramphenicol; the cells were converted to spheroplasts to increase accessibility of the cytoplasmic membrane; and the spheroplasts were treated with ^{35}S-labelled acetylmethionyl methylphosphate sulphone (AMMP), which reacts with free amino groups (of protein and phospholipid) on the cell surface but does not penetrate cell membranes[33]. The spheroplasts were then washed and gently disrupted by osmotic lysis; and the membrane–polysome complexes were separated, by sedimentation in a sucrose gradient or by gel filtration, from the free membrane fragments and the free polysomes.

The polysomes from the membrane–polysome complexes were freed of membrane by washing with detergent, and they were found to retain about 6% of the initial label in the complexes (2% of the total incorporation). More recently, use of ^{125}I-diazoiodosulphanilic acid, a non-penetrating reagent that labels only tyrosine and histidine residues and not phospholipid, has increased about fourfold the proportion of cellular label that is recovered on polysomes[34]. This label on the polysomes (from either reagent) is evidently attached via peptidyl-tRNA: it was released by low Mg^{2+}; subsequent cleavage by dilute alkali decreased the average molecular weight by about that of tRNA; and the label was also released by puromycin, or by chain completion *in vitro*. Moreover, the product of chain completion, as expected, had an average molecular weight about twice that of the material released by puromycin. (This growth of the labelled molecules is particularly cogent evidence, for it excludes the possibility that the small amount of label in the purified polysomes was merely contamination.) Finally, the demonstration of cotranslational secretion was completed by identifying a periplasmic enzyme, alkaline phosphatase, among the products of completion of the extracellularly labelled growing chains[32].

Using the same procedure, W. P. Smith in this laboratory has since demonstrated cotranslational secretion of several other proteins, in both Gram-positive and Gram-negative bacteria: α-amylase of *Bacillus subtilis*[34], the toxin of *Corynebacterium diphtheriae*[35], and penicillinase of *Bacillus licheniformis* (unpublished data, in collaboration with J. O. Lampen). Thus it seems that simultaneous translation and extrusion is a general (although not necessarily an exclusive) mechanism of protein secretion in bacteria.

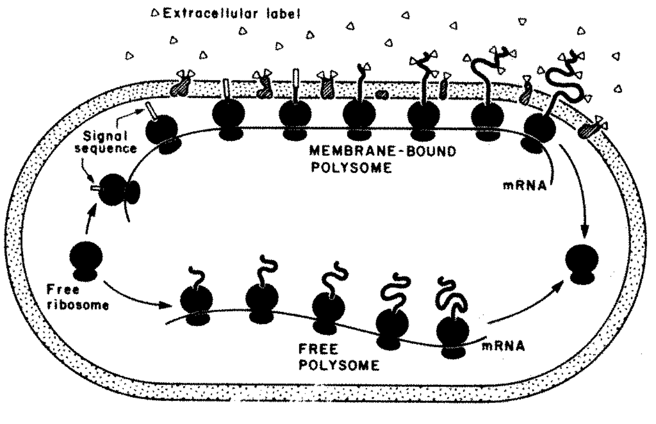

Fig. 1 Use of extracellular labelling of nascent chains to demonstrate secretion of proteins across membrane as growing chains. (Adapted from refs 32, 34.)

Recently, some intrinsic membrane proteins have also been shown to enter the membrane during chain growth, as might be expected from the demonstration[22-26] that such proteins are formed as cleavable precursors with a hydrophobic signal sequence. Thus incomplete protein chains have been recovered from the outer membrane of pulse-labelled *E. coli* cells in steady-state growth[36]. Moreover, in animal cells ingenious experiments have shown that during the insertion of a viral protein into the membrane of the endoplasmic reticulum, the protein can be glycosylated, on the intraluminal surface, only during the early part of chain growth and not later[37,38].

Significance of membrane-associated ribosomes in bacteria

The demonstration of cotranslational secretion (and incorporation into membrane) has incidentally settled the long-standing question of the functional significance of the association of ribosomes with membrane in bacteria. In thin sections of these cells, unlike eukaryotic cells, it has not been possible to distinguish membrane-bound and free polysomes by electron microscopy, because the population of ribosomes is too dense. Hence even though membrane fragments in bacterial lysates have long been observed to contain ribosomes, it has not been certain whether the attachment is functional or is due to artificial trapping. A functional role, like that observed earlier in eukaryotes, is now established.

A functional attachment was also suggested by the finding that the membrane-bound polysome fraction of *E. coli* produces a somewhat greater abundance of a secretory protein (alkaline phosphatase) than does the free fraction[39]. Similarly, Randall and Hardy[40] prepared membrane–polysome fractions whose products were more enriched in several secreted or incorporated proteins, while containing very little of a cytoplasmic protein (elongation factor EFTu). In our laboratory W. P. Smith has achieved a more complete separation, yielding virtually exclusive synthesis of alkaline phosphatase, α-amylase, penicillinase, and diphtheria toxin on membrane-bound polysomes of various organisms, while a cytoplasmic protein (elongation factor G of *E. coli*) was made exclusively on free polysomes.

Synthesis and processing of precursors

Polysomes derived from membrane–polysome complexes have regularly yielded precursors of the secreted or membrane proteins described above, and so they appear to be a good source of precursors and intermediates in protein formation. Precursors have also been formed in purified systems directed by DNA[18,20], and in toluene-treated cells[41]. A number of these products have been shown to be more hydrophobic than the mature proteins.

The penicillinase of *Bacillus licheniformis*, formed by chain completion on membrane-derived polysomes, is a particularly interesting example, since four molecular forms can be recovered (unpublished experiments, with W. P. Smith). One of these evidently corresponds to the familiar extracellular product of MW 29,000 (29K), and another to a membrane-bound form of 33K, which has been reported to contain phospholipid[42,43]. In addition, our 33K product could be separated on decylagarose into a highly hydrophobic and a hydrophilic fraction; and only the hydrophobic 33K fraction contained ^{3}H-glycerol (presumably in phospholipid) when derived from cells carrying this label. Finally, an even larger form (see also ref. 20) was also recovered, and it was more hydrophobic than the secreted product, as expected of a precursor. Evidently secreted proteins in bacteria can go through successive cleavages, like the preproproteins that have been recognised in eukaryotic cells as precursors of protein hormones[44,45]. In addition, and unexpectedly, the successive cleavages of penicillinase can all occur during chain growth. Interestingly, diphtheria toxin also goes through more than one cleavage: one on leaving the bacterial cell[35], and another on entering the target mammalian cell[46].

Table 1 Amino acid sequence of the N-terminal signals of precursors of some secreted (S) or membrane (M) proteins

Complete sequence	25	24	23	22	21	20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	Refs
Eukaryotes																										
1. Pre-immunoglobin κ-type light chain (mouse) (S)				Met	Asp	Met	Arg	Ala	Pro	Ala	Gln	Ile	Phe	Gly	Phe	Leu	Leu	Leu	Leu	Phe	Pro	Gly	Thr	Arg	Cys↓Asp	29
2. Pre-lysozyme (hen oviduct) (S)								Met	Arg	Ser	Leu	Leu	Ile	Leu	Val	Leu	Cys	Phe	Leu	Pro	Leu	Ala	Ala	Leu	Gly↓Lys	30
3. Pre-parathyroid hormone (bovine) (S)	Met	Met	Ser	Ala	Lys	Asp	Met	Val	Lys	Val	Met	Ile	Val	Met	Leu	Ala	Ile	Cys	Phe	Leu	Ala	Arg	Ser	Asp	Gly↓Lys	31
4. Preglycoprotein (vesicular stomatis virus) (M)										Met	Lys	Cys	Leu	Leu	Tyr	Leu	Ala	Phe	Leu	Phe	Ile	His	Val	Asn	Cys↓Lys	27
Prokaryotes																										
1. Prelipoprotein (*E. coli*) (M)						Met	Lys	Ala	Thr	Lys	Leu	Val	Leu	Gly	Ala	Val	Ile	Leu	Gly	Ser	Thr	Leu	Leu	Ala	Gly↓Cys	22
2. Pre-β-lactamase (*E. coli*) (S)			Met	Ser	Ile	Gln	His	Phe	Arg	Val	Ala	Leu	Ile	Pro	Phe	Phe	Ala	Ala	Phe	Cys	Leu	Pro	Val	Phe	Ala↓His	21
3. Precoat protein (phage fd) (M)			Met	Lys	Lys	Ser	Leu	Val	Leu	Lys	Ala	Ser	Val	Ala	Val	Ala	Thr	Leu	Val	Pro	Met	Leu	Ser	Phe	Ala↓Ala	25

(Residues are numbered leftward from the cleavage site, ↓)

The enzymes responsible for the cleavage of signal segments are being investigated in several laboratories; so far the distinction between specific and nonspecific cleavage is hard to establish. We shall not review this topic in detail. With Gram-negative bacteria both inner[26] and outer[18] membrane fragments have been found to carry out the reaction in various systems; in some the presence of detergent is required[26,47,48]. Cleavage can occur with some precursors only during formation, but with others even after completion of the protein chain.

Neither the fate of the cleaved segments nor the reason for their removal is known. Perhaps retention of a signal would cause the secreted protein to reattach to the membrane, or, more generally, to have less freedom of motion and specificity of interaction.

Genetic studies

The genetic manipulation of bacteria, to yield mutants or recombinants altered in various functions, provides a powerful tool for the study of the secretory process. Thus alterations in the signal sequence may identify its essential features. In addition, mutants altered either in membrane components, the ribosomes, processing activities, energy transduction, or other, unknown factors should throw light on the mechanism of secretion.

Using an elegant technique for gene fusion[49,50] Beckwith, Silhavy and associates[51–56] have isolated strains of *E. coli* in which an *N*-terminal sequence of a secreted or an incorporated protein, including its signal segment, replaces the *N*-terminus of a cytoplasmic protein, β-galactosidase (for details, see ref. 54). Those hybrid proteins that contained the *N*-terminus of the interpreted (outer membrane) protein, phage λ receptor, were found to be localised either in the cytoplasm or in the outer membrane of the cell, depending on the length of the membrane protein component[51]. In contrast, hybrids with the periplasmically secreted maltose-binding protein were localised in the cytoplasmic membrane, and not in the periplasm, even when they contained almost the entire maltose-binding protein (including its signal segment)[56]. Thus secretion seems to depend not only on the signal sequence but also on the subsequent sequence: evidently some peptide sequences cannot pass through the secretory channel[56].

In these experiments the secretory hybrids that stick in the membrane were also found to interfere with the export of certain normal outer membrane proteins, which then accumulate in the cells in their precursor form[56]. This finding provides evidence that secretory and membrane proteins initially interact with membranes in the same way, that is, enter at the same site(s); presumably a later, probably hydrophobic 'stop' signal in the non-secreted proteins causes them to leave the channel and become embedded in the lipids of the membrane[55]. Such a second signal was already postulated earlier[27,38], on the basis of the similarity of the initiating signal sequences in the two kinds of proteins.

Other interesting classes of mutants have also been obtained. (1) In certain hydrids the function of the signal sequence has been eliminated by partial deletions, and also by various single amino acid substitutions (J. Beckwith, personal communication); the precursor then accumulates in the cytoplasm[53,55]. Moreover, when mutations of either class are transferred genetically to the normal secreted or incorporated proteins, precursors of these proteins also accumulate in the cytoplasm. These results provide direct evidence for the essential role of the signal sequence in the initiation of secretion; in addition, the deletions define the minimal length of an effective sequence. (2) The effects of some of these mutations could be reversed by a suppressor mutation; and it is interesting that this mutation appears to map in a ribosomal protein (S. Emr and T. Silhavy, personal communication; see ref. 54). (3) Another mutation, presumably in a membrane component, greatly decreases the secretion of various periplasmic proteins, and also the incorporation of outer membrane proteins[57]. (4) Finally, a point mutation in the signal sequence of the lipoprotein precursor prevents its cleavage but, nevertheless, allows its incorporation into the outer membrane (although the altered *N*-terminus can no longer be linked to the peptidoglycan)[23]. It thus seems that cleavage is not required for export of a protein to the outer membrane.

Source of energy for cotranslational secretion

The hydrophobicity of the signal segment might lead to its spontaneous insertion between lipid molecules in the membrane. However, the source of energy for the extrusion of the subsequent, largely hydrophilic nascent chain is obscure. Figure 2 illustrates several possibilities.

(a) Chain elongation on an attached ribosome: Part of the energy expended on chain elongation on the ribosome might be used to push the chain through the membrane (Fig. 2*a*). This mechanism would require that the ribosome have a direct attachment to the membrane, to serve as a fulcrum. Purified microsomes from animal cells[59,60], and chloroplasts from plant cells[61], are thought to have such an attachment, since release of the nascent chains from the ribosomes by puromycin did not release the ribosomes from the membrane, unless the salt concentration was elevated or a cytoplasmic detachment factor was provided. However, with purified membrane–polysome complexes from bacteria we found that puromycin released the ribosomes[34,62], even in ionic conditions (high Mg^{2+}, low K^{+}) that might be expected to stabilise a weak association[62]. This finding suggests that ribosomes in bacteria are attached to the membrane solely by their nascent chains. If so, the ribosomes cannot push these chains through the membrane. However, an alternative explanation for ribosome release by puromycin is not rigorously excluded: loss of a growing chain embedded in the membrane might cause a conformational change, either in the ribosome or in a membrane protein, that would disrupt a direct attachment of ribosome to membrane.

(b) Folding of the extracellular chain: After entry of the relatively hydrophobic initial segment into the lipid of the membrane a few residues of that segment, or of a later one in a loop[63], might somehow be forced into the aqueous phase on the far side (Fig. 2*b*); the spontaneous folding of the protruding chain might then progressively pull the rest of the chain after it[34,63]. However, when the chains protruding from protoplasts were excised, by treatment with pronase, the ribosomes remained attached to the membrane[64]; hence they are not held by an extracellular 'knot'. Moreover, alkaline phosphatase of *E. coli* can still be transferred across the membrane even after extensive modification (by mutation, incorporation of amino acid analogues, or premature termination yielding fragments as small as 10%: reviewed in ref. 65). Extracellular folding therefore seems an unlikely source for the energy of secretion.

(c) Embedding of the ribosome in the membrane: If the ribosome, rather than the protein chain, were embedded in the membrane (Fig. 2*c*) the nascent chain leaving it might already be extracellular. However, in electron micrographs membrane-bound ribosomes do not appear to be embedded; moreover, we have shown that the membrane protects part of the growing chain from proteolytic digestion[64].

These experiments on protection from digestion have shed further light on the secretory process, for they have shown that the protected length is uniform for many nascent chains traversing the membrane, and they have defined that length. In these experiments cells of *Bacillus subtilis* were pulse-labelled with a mixture of radioactive amino acids, chilled, and converted to protoplasts, and pronase was used to 'shave' the protruding chains. The membrane (plus ribosomes) protected a length of 49 residues for 50% of the chains[64]. (Most of the rest were longer, and of varying length; they were presumably being folded into the membrane.) In control experiments, membrane-free ribosomes protected a uniform length of 28 amino acid residues, as noted earlier with mammalian ribosomes[66,67]. The difference between 49 and 28 (21 residues) may be taken as the approximate size of the secreted chain surrounded by membrane. As an extended polypeptide chain this number of residues would have the same length, 75 Å, as the commonly observed thickness of bacterial membranes.

(d) An active secretory machinery in the membrane: The most plausible model for cotranslational secretion appears to be one in which an organised membrane structure surrounds the secreted chain and transports it, unidirectionally, by transducing metabolic energy (Fig. 2*d*). As in the active transport of small molecules, the energy could be derived from the proton-motive force or from intracellular high-energy phosphate. Such an active carrier would differ from the passive tunnel that has been postulated previously[15,60,63], but it shares the possibility that the 'channel' is induced, by the signal sequence, to assemble in the fluid membrane from mobile components[15,60].

This model is supported by several lines of evidence. Thus a uniform apparatus would be expected to enclose a uniform length of nascent chain; and the experiments with pronase described in the preceding section yielded precisely this result[64]. Moreover, any machinery for actively transporting a chain, unlike a passive tunnel, would be expected to hold the chain firmly; and indeed, after elimination of the extracellular chains on protoplasts by pronase the subsequently isolated membrane-polysome complexes retain their ribosomes, even when incubated at 37 °C for 30 min[64]. Finally, the postulated apparatus would presumbly contain specific proteins; and in the membrane from the membrane–ribosome complexes of *B. subtilis* we have observed two major protein bands that are absent from the free membrane fraction (D. Marty, P.-C. T. and B. D. D., unpublished).

Nevertheless, the significance of the extra proteins is not certain. Two additional membrane proteins have been observed in the ribosome-bearing fraction of the endoplasmic reticulum in animal cells and have been termed 'ribophorins'[68,69], because they are believed to mediate direct binding of ribosomes. Although our evidence argues against such a direct attachment in bacteria, we have noted that a conformation-dependent attachment would be difficult to exclude; and such an attachment, continuous with a passive membrane channel, would also be consistent with the lines of evidence cited in the preceding paragraph. The question of an active or a passive channel for protein secretion is thus still unsettled.

Alternative mechanisms for export and insertion

Cotranslational secretion, although widespread, is not the only mechanism for protein export. The suggested[70–72] alternative of secretion (or incorporation into membrane) after synthesis in the cytosol, has been demonstrated in both prokaryotes and eukaryotes, with or without formation of a cleavable precursor. We shall only briefly review some key observations.

In prokaryotes cytoplasmic synthesis, in precursor form, has been demonstrated for subunit *A* of cholera toxin[73] (which is later combined with subunit *B* and secreted). Synthesis of a cytoplasmic precursor followed by insertion of a cleaved product into the cytoplasmic membrane has been reported[74], but disputed[75], for a phage coat protein. In eukaryotes, cytoplasmic synthesis of a precursor has been observed for a subunit of the ribulose-bisphosphate carboxylase of the chloroplast interior[76–80], and for several subunits of ATPase[81] and of cytochrome oxidase[82,83] of the mitochondrial inner membrane. The oxidase subunits are formed by several cleavages of a single large precursor, while other subunits of the same transmembrane protein are made within the mitochondrion[82,83]. It is not clear why the cleavable signal segment of certain proteins fails to direct the polysome to become membrane-bound, or what role this segment plays in triggering[84] the interaction of the completed protein with the membrane. Nor is it clear why ATP is required for the incorporation of some cytoplasmically synthesised mitochondrial proteins[85].

Other proteins are secreted or incorporated in the form in which they are synthesised, that is without the need of a precursor form. In bacteria these include colicin E1 and E3[86], and several products of a sex factor[87]: whether these proteins are synthesised by membrane-bound or by free polysomes is not known. In eukaryotes the best example[88,89] is ovalbumin (in contrast to other proteins secreted by the hen oviduct[30,39]). Ovalbumin is particularly interesting: its *N*-terminal region is not highly hydrophobic[88], and a hydrophobic segment in the middle of the molecule may serve as the signal. This conclusion was based on the finding that secretion of another protein (from a precursor) into vesicles *in vitro* can be competitively inhibited by this segment, either exposed in the incomplete or the denatured chain[91], or isolated as a fragment from the molecule[92].

Cytoplasmic synthesis preceding secretion seems to be incompatible with provision of energy by a membrane-attached ribosome; it would be compatible either with embedding of the folded protein in the membrane (followed, for secretory proteins, by release on the other side), or with insertion of the unfolded chain into an active machinery of transport. In addition, if ovalbumin initiates insertion at an internal sequence it would have to be drawn through the membrane as a long hairpin, or as a folded protein; this process also seems incompatible with being pushed by the ribosome through a passive channel.

Membrane morphogenesis

Eukaryotes (and some prokaryotes) have more than one kind of membrane in the same cell, thus raising the broad problem of morphogenetic specificity. Specific signal sequences may well determine the choice of different membranes by different growing proteins—a possible role that enhances interest in these sequences. A good model system with which to study this

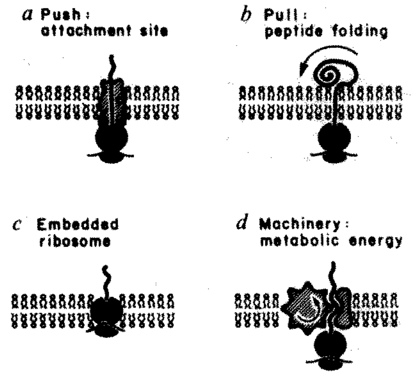

Fig. 2 Models for cotranslational chain extrusion across membrane. *a*, The energy derives from the GTP hydrolysis required for chain elongation on the ribosome; this model requires a firm ribosomal attachment to membrane and a fixed channel in the membrane. *b*, Spontaneous extracellular folding of nascent peptide pulls the chain through the membrane. *c*, Ribosomes are embedded in the membrane, releasing their nascent chains directly to the farther side. *d*, The membrane itself provides a machinery for transducing metabolic energy into unidirectional transfer of the chain. (Adapted from refs 58, 62.)

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possibility may well be the formation of the inner and the outer membrane of Gram-negative bacteria. Although the proteins of the outer membrane may be secreted across the inner membrane and only then incorporated, after crossing the peptidoglycan layer, it seems more likely that they selectively enter the junctions (adhesion sites[93]) between the two membranes and then move outwards in the two-dimensional fluid of the membrane. Thus matrix protein of the outer membrane is localised initially at adhesion sites[94], and bacteriophage λ receptor protein only at the cell septum[95].

Among the proteins in the periplasm, secretion is reported to occur only during cell division for galactose- and maltose-binding proteins[96,97], but throughout the cell cycle for alkaline phosphatase[96]. These differences provide further evidence for diversity in the mechanisms of transfer.

Discussion

In the past 8 years, since the discovery of the *N*-terminal signal sequence of an immunoglobulin precursor, our knowledge of the mechanism of protein secretion has progressed a good deal. The process is very similar in bacteria and in eukaryotic cells, except that after peptide release, by puromycin, ribosomes remain attached to membrane in preparations from animal cells but not in those from bacteria. This difference might reflect a basic difference in mechanism, perhaps associated with a greater stability of the machinery of secretion in animal cells. On the other hand, the mechanisms might be the same, one of the two observations failing to reflect the physiological process.

The central problem of protein secretion is now the identification of the basic molecular mechanism that ensures unidirectional transfer of the growing chain. The available evidence seems to point towards a specific machinery for active transport of proteins across the membrane; but direct attachment of the ribosome, permitting it to supply the energy, is not rigorously excluded. The decision between these mechanisms must await further information, either on the nature of the contact between ribosome and membrane, or on the source of the energy for transfer of the growing chain. And among other aspects of the process, we do not know whether the signal sequence is first inserted into lipid linearly[15] or as a loop[28,63]; or whether the channel surrounding the chain exists preformed or is induced to assemble from separate molecules in the membrane. Finally, since at least some secreted and some incorporated proteins seem to enter in the same way, the mechanism responsible for their subsequent divergence, presumably triggered by a second, later signal segment, remains to be determined. A possible model for this 'stop' signal is the anchoring of an erythrocyte membrane protein[98], and a penicillin-binding enzyme of the inner membrane of *B. stearothermophilus*[99], by a cleavable COOH-terminal segment. In the latter protein a rather hydrophobic sequence has been determined for the cleaved segment (D. J. Waxman and J. Strominger, personal communication).

It seems reasonable to expect that genetic studies will shed much light on these problems. Another approach is provided by the use of conditions that interfere with secretion by decreasing the fluidity of the membrane[100–102].

In bacteria the presence of a multi-layered rigid envelope presents a special problem for secretion of proteins into the medium: it is not known whether these proteins pass through the successive layers, or through specialised regions of the cytoplasmic membrane that are exposed to the exterior. In addition, the segregation of different proteins into the inner and the outer membrane of Gram-negative bacteria presents a problem of membrane morphogenesis analogous to that of eukaryotic cells.

Knowledge of signal sequences for secretion has recently found practical applications. For example, to increase the yield of insulin in recombinant bacteria the gene for insulin has been fused with the beginning of the gene for the *E. coli* periplasmic *β*-lactamase; the resulting hybrid protein is secreted, and it can then be recovered by osmotic shock and cleaved to yield the hormone[103]. In addition, the bacterial mechanism of secretion can evidently recognise a mammalian signal, since bacteria containing the ovalbumin gene secrete its product[104,105].

The experiments described here illustrate the continuing value of bacteria as model cells, in an era that has seen a large shift away from this approach to cell biology.

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ARTICLES

Sr isotopic fractionation in Ca–Al inclusions from the Allende meteorite

P. J. Patchett*

Department of Geology and Mineralogy, Parks Road, Oxford, UK

True relative Sr isotopic compositions, determined by double spiking on Ca–Al inclusions from the Allende meteorite show up to 1.5‰ per mass unit mass fractionation relative to the Earth and bulk chondrites. All abnormal inclusions are light-isotope enriched. A lack of isotopically heavy Sr in inclusions would place constraints on the time, place and mechanism of origin of these objects.

A FEATURE of much of recent data[1–4] on isotopic anomalies in meteorites is that an arbitrary value for a certain isotopic ratio has been used to correct for mass fractionation in the mass spectrometric analysis. This means that the true isotopes responsible for any anomalous isotopic ratio cannot be uniquely identified, and several interpretations of mixing of nucleosynthetic components are possible[5,6]. The double spike isotope dilution technique, however, corrects for laboratory fractionation, and allows a full comparison between the isotopic compositions of samples[7,8]. The method leads to clear identification of the isotopes involved in nucleosynthetic anomalies, and also any natural mass fractionation processes which occurred in the sample before analysis[9,10].

Two spike solutions, enriched in ^{87}Rb, ^{84}Sr and ^{87}Sr, and differing only in Rb/Sr ratio, were both calibrated in quadruplicate against NBS 987 Sr. The composition for NBS 987 of $^{88}\mathrm{Sr}/^{86}\mathrm{Sr} = 8.37521$, $^{87}\mathrm{Sr}/^{86}\mathrm{Sr} = 0.71014$ and $^{84}\mathrm{Sr}/^{86}\mathrm{Sr} = 0.056584$ was assumed. The spike composition was calculated by means of the double spike mathematical solution[8], where NBS 987 constituted the 'spike' for the determinations, and the eventual spike constituted the 'sample'. As the composition of NBS 987 is a very poor spike for this purpose, the error propagations are large; resulting spike compositions are given in Table 1. The spike allows inter-sample comparison of total isotopic composition, but cannot yield 'absolute' values for external application, because no absolute calibration standard is yet available.

Samples were handled with stainless steel tools in clean room conditions. An HF/HNO_3 bomb dissolution was used, and great care was applied to ensure total solution of the sample after this stage, first in HNO_3 and then in HCl. The total sample was then divided into aliquots in HCl solution and spiked. Completely separate sets of equipment were used for spiked and unspiked

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*Present address: US Geological Survey, Denver Federal Center, Denver, Colorado 80225.

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Table 1 Rb–Sr spike compositions

Spike	Rb/Sr	^{85}Rb/^{87}Rb	^{88}Sr/^{87}Sr*	^{86}Sr/^{87}Sr*	^{84}Sr/^{87}Sr*
Rb–Sr-1	0.30005	0.008908	0.088155 ±12	0.0097440 ±14	0.95547 ±40
Rb–Sr-2	0.015265	0.008908	0.088130 ±12	0.0097365 ±14	0.95541 ±40

* Errors are two standard errors of the mean of four calibrations.

sample solutions, thus eliminating the possibility of cross-contamination. Ion-exchange separation was two-stage for Sr in PTFE columns; Rb fractions were further purified[11]. Blanks were <0.1 ng Rb and <0.2 ng Sr; for the small samples ALL-24, 40 and 41, where the first, larger column was omitted, Rb and Sr blanks were <0.03 ng.

Mass spectrometry was performed using a VG Micromass 30, fitted with a Cary 401M amplifier since July 1978. Ion currents corresponding to the most abundant isotopes were at a level of $1.5\text{-}3\times10^{-11}$ A. Isotopic data were collected and reduced as blocks of 10 scans over the mass range; 25–50 such blocks constitute a run. In several cases almost all the Sr on the filament was used in the analysis, and progressive in-run mass fractionation of up to 2% per mass unit was observed; no evidence was seen in any of the runs for detectable departures from linear first-order mass fractionation (compare ref. 10 for Ca).

^{87}Sr was used as reference isotope for data collection and the double spike calculation. The equations for the double spike solution[8] were used to propagate errors in a mathematically rigorous way, using a summation of partial differentials. Only errors in spike composition, which can be disregarded for inter-sample comparison, are excluded from the final uncertainties given in Table 2. Inclusion of this component would result in a ~30% increase in errors on isotopic ratios.

Mean values for 10 runs of NBS 987 over the period of study were $^{87}\text{Sr}/^{86}\text{Sr} = 0.70995\pm3$ $(2\sigma_m)$, $^{84}\text{Sr}/^{86}\text{Sr} = 0.056512\pm16$ $(2\sigma_m)$, when normalised to $^{88}\text{Sr}/^{86}\text{Sr} = 8.37521$. Baselines were always constant over the spectrum to better than 0.2%, and the difference between our values and NBS values represents a bias in amplification of peaks. Results from other laboratories (see refs 3, 12) show comparable variations, particularly in ^{84}Sr/^{86}Sr. As our amplifier tends to overemphasise larger peaks, then the results obtained by double spike analysis of NBS 987 fall around $^{88}\text{Sr}/^{86}\text{Sr} = 8.378$, $^{84}\text{Sr}/^{86}\text{Sr} = 0.05649$, instead of the 8.375 and 0.05658 used for spike calibration. The amplifier contributes a small bias to the analyses towards higher ^{88}Sr/^{86}Sr and lower ^{87}Sr/^{86}Sr and ^{84}Sr/^{86}Sr.

Because this study reports small differences in Sr isotopic composition for Allende inclusions, we need to demonstrate beyond all doubt that no inter-sample bias has occurred. There are only three differences in the way in which samples have been treated: (1) ALL-24, 40 and 41 were passed through only one ion-exchange column instead of two; (2) whole meteorite and Allende matrix samples were analysed with spike solution Rb–Sr-1. Rb–Sr-2 was used for mantle nodules and Allende inclusions; (3) there was large variation of the sample Sr/spike Sr ratio, from 0.07 to 3.09 (Table 2). A value of 0.5 is considered optimum.

Table 2 shows that ALL-24, 40 and 41 are quite uncritical for the overall result of this study, and the minor difference in chemical procedure is therefore insignificant. The test analyses of NBS 987 given in Table 2 are crucial in eliminating items 2 and 3 as sources of bias. These tests were all processed using the same run data for unspiked NBS 987, so that they compare closely the results of four double spike runs. Test 1 was made using spike Rb–Sr-2, and its agreement with the other three tests, made with Rb–Sr-1, shows that there is no bias connected with which of the two spike solutions is used. Tests 2, 3 and 4 were made with Rb–Sr-1, where the sample Sr/spike Sr ratio varied from 0.19 to 1.09. As the results are identical within errors, it is concluded that no bias is associated with change in the sample Sr/spike Sr ratio. Additionally, Sr compositions close to those of NBS 987 were determined for terrestrial analyses where the sample Sr/spike Sr ratio ranged up to 3.09 (Table 2). The small bias introduced by the amplifier is approximately constant for all analyses, in spite of variation of peak sizes between different spiked runs. Hence all the analyses reported here are closely comparable in every respect.

Terrestrial and bulk chondritic Sr

The analyses of chips of four terrestrial mantle nodules and of four bulk chondrites are presented in Table 2. Rb was not analysed for these samples. The Sr isotopic compositions plot in a tight group with the four NBS 987 analyses, and together define the Earth plus bulk chondrite field of Fig. 1.

Allende Sr

All samples were extracted from a single 450 g individual. Diameters of Ca–Al inclusions (CAI) range from 15 down to 1 mm, but none smaller than 2.5 mm were analysed. All fine-grained CAI are pink except ALL-40, which is white; ALL-24, 30, 72 and 76 are colour-zoned, with white margins. Thin sections show that of the coarse-grained CAI, ALL-4, 6, 37, 46, 56 and 70 are clearly type B (ref. 13), while ALL-7 and 68 are apparently intermediate between A and B; ALL-41 and 49 were not examined.

Plotting of the results (Table 2) on an Rb–Sr isochron diagram (not given) shows a scatter of points lying below a 4.6 Gyr line, closely similar to earlier studies[14,15] and possibly reflecting a relatively recent disturbance[12,14–16]. In terms of $^{84\text{-}86\text{-}88}$Sr isotopic composition, the matrix samples plot close to the Earth/bulk chondrite field (Fig. 1), while the CAI define a continuous trend towards lower ^{88}Sr/^{86}Sr and higher ^{84}Sr/^{86}Sr. This trend is completely consistent with the predicted mass fractionation path, and regression of the data gives a slope of -1.05 ± 0.18 (2σ), where MSWD = 2.3. Within error, the same line is obtained if either the coarse- or the fine-grained CAI are regressed alone. A second chip of the most abnormal sample (ALL-76, a fine-grained pink CAI) was dissolved, confirming its general position. The analysis is not an exact repeat because the inclusion was not powdered; indeed, the ^{87}Sr/^{86}Sr ratios (Table 2) and model ages of the two chips are very different probably because of the recent isotopic disturbance. The white margin of ALL-76 has an Sr composition lying approximately half way between that of the pink interior and the Allende matrix (Fig. 1). ^{85}Rb/^{87}Rb ratios for three samples (Table 2) are not regarded as deviating significantly from the accepted terrestrial value of 2.593, because mass fractionation cannot be controlled or reproduced in the runs.

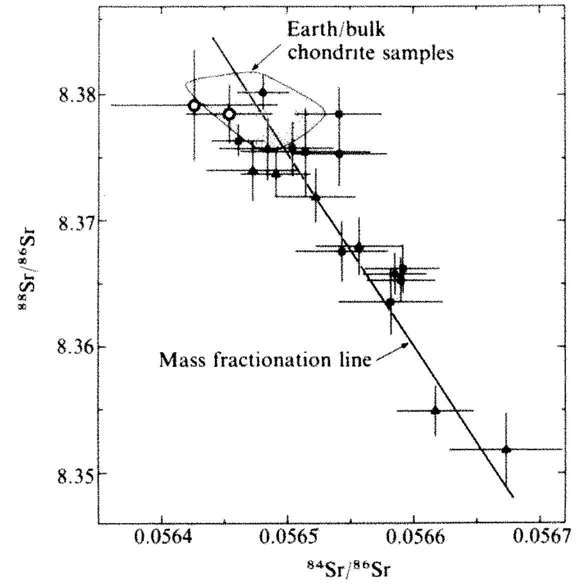

Fig. 1 ^{88}Sr/^{86}Sr–^{84}Sr/^{86}Sr plot for the double-spike analyses reported here. Shaded area includes four NBS 987 analyses, four terrestrial mantle nodules and four bulk ordinary chondrites. All marked points, with errors at 2σ, are from the Allende meteorite, ○, Matrix; ■, coarse-grained CAI; ▲, fine-grained CAI.

Discussion

The results for terrestrial, bulk chondrite and Allende matrix samples demonstrate that the accretion regions of the Earth, ordinary chondrites and Allende matrix were essentially uniform in Sr isotopic composition.

For the Allende CAI samples, an origin for the variation of ^{88}Sr/^{86}Sr and ^{84}Sr/^{86}Sr in the recent isotopic disturbance of the meteorite[12,14–16] can be ruled out because this would only lead to homogenisation of ratios, and could not by itself produce an apparent mass fractionation. Heavy element fission can be ruled out because ^{84}Sr and ^{86}Sr are shielded from all but direct yields. A neutron flux[17] affecting all Solar System Sr except that in some CAI could explain a pattern in which Solar System ^{84}Sr became depleted and Solar System ^{88}Sr enriched relative to the CAI. However, the neutron-capture cross-sections[5,18] show that in a neutron irradiation, burning-out of ^{84}Sr would predominate over all other effects; one-to-one correlation of ^{84}Sr and ^{88}Sr anomalies is almost certainly impossible.

The pattern of Fig. 1 can be produced by certain combinations of nucleosynthetic components. In the case of strontium, ^{84}Sr is thought to be of 100% p-process origin, ^{86}Sr and ^{87}Sr are s-process only (except for a minor contribution to ^{87}Sr from ^{87}Rb), while ^{88}Sr may be 90% s-process plus 10% r-process[5]. The compositions of the abnormal CAI relative to Solar System Sr could be explained by any of excess p plus deficient r, excess p plus smaller excess s, or deficient r plus smaller deficient s. Such nucleosynthetic explanations are unlikely for two reasons. First, in such a model, closely correlated effects are needed in partly independent components, to produce the slope of −1 in Fig. 1. Apart from this *a priori* improbability, Papanastassiou *et al.*[3] have discovered, in two CAI, a nucleosynthetic anomaly in Sr with quite different characteristics. The Sr from these two CAI cannot be plotted in Fig. 1, because of the necessity for normalisation in the runs[3], but the points must lie on mass fractionation lines to the left of that in Fig. 1. Perhaps there are anomalies of nucleosynthetic origin showing good correlation of ^{88}Sr/^{86}Sr and ^{84}Sr/^{86}Sr effects in many inclusions (those of this study), and an additional, uncorrelated component present in only a few (EK1-4-1 and C1), but this seems highly unlikely.

The excellent fit of the data points to a mass fractionation line suggests strongly that mass fractionation is the physical explanation for the variation. Accepting this, the striking feature is that all the samples are either normal or enriched in light isotopes. In contrast, Si, Mg and O from CAI show either heavy-isotope enrichment, or both positive and negative δ-values[19–23]. Although another Allende stone might show heavy-Sr enrichment in its CAI, the observed consistent light-Sr enrichment requires explanation. No clues are derived from other parameters, because the position of points on Fig. 1 is uncorrelated with inclusion type, inclusion size, major mineralogy, Sr content and Rb content. The fractionation has affected both coarse- and fine-grained CAI.

It seems likely that these CAI formed from a vapour which was isotopically lighter than normal Solar System Sr, because mass fractionation effects always lead to formation of an isotopically heavy solid and an isotopically light vapour[24]. If the rate-determining and fractionating step in the Sr accretion process was diffusion of Sr into pre-existing solid Ca–Al objects, then light isotopes might diffuse faster than heavy isotopes, and so produce isotopically light cores plus isotopically heavy rims from normal vapour. If the outer zones were later removed by volatilisation, then overall isotopically light CAI would result,

Table 2 Sr double-spike and Rb data

Sample	mg*	sa/sp†	Rb p.p.m.	Sr p.p.m.	^{87}Rb/^{86}Sr‡	^{85}Rb/^{87}Rb	^{88}Sr/^{86}Sr‡	^{87}Sr/^{86}Sr‡	^{84}Sr/^{86}Sr‡
NBS 987 Sr									
Test 1	—	0.68	—	—	—	—	8.3792±29	0.71007±17	0.056491±43
Test 2	—	1.09	—	—	—	—	8.3776±23	0.71000±14	0.056502±35
Test 3	—	0.61	—	—	—	—	8.3775±23	0.70999±14	0.056503±34
Test 4	—	0.19	—	—	—	—	8.3798±24	0.71009±14	0.056487±36
Terrestrial mantle nodules§									
AJE 62	752	0.51	—	9.667	—	—	8.3806±34	0.70353±20	0.056467±51
BD 2344	756	3.09	—	42.146	—	—	8.3800±29	0.70490±17	0.056491±44
BD 2384	497	1.60	—	33.491	—	—	8.3813±60	0.70557±36	0.056469±91
JJG 846	642	2.45	—	45.049	—	—	8.3771±20	0.70470±12	0.056479±30
Bulk ordinary chondrites‖									
Parnallee LL3	702	1.02	—	10.989	—	—	8.3812±37	0.76247±24	0.056465±57
Richardton H5	557	0.46	—	9.804	—	—	8.3800±39	0.75337±25	0.056462±58
Barwell L5	995	0.58	—	7.527	—	—	8.3804±21	0.74529±13	0.056435±32
Bruderheim L6	856	0.83	—	9.346	—	—	8.3788±24	0.74667±15	0.056516±36
Allende C3V‖ matrix									
Anal. 1	813	0.89	0.921	13.428	0.1986±2	—	8.3792±44	0.71209±27	0.056426±67
Anal. 2	516	0.75	0.870	13.247	0.1901±3	—	8.3785±23	0.71126±14	0.056454±35
Allende coarse-grained Ca–Al inclusions									
ALL-4	98	0.59	2.131	111.75	0.05513±8	—	8.3755±34	0.70174±20	0.056514±51
ALL-6	96	0.56	0.953	142.97	0.01925±2	—	8.3636±27	0.69893±16	0.056582±41
ALL-7	33	0.60	0.646	161.72	0.01153±4	—	8.3662±19	0.69878±11	0.056590±30
ALL-37	72	1.10	1.285	240.57	0.01544±2	—	8.3754±25	0.69934±15	0.056541±38
ALL-41	17	0.43	2.016	178.59	0.0326±2	—	8.3758±21	0.70075±13	0.056504±33
ALL-46	32	0.62	4.309	124.20	0.1003±5	—	8.3676±24	0.70456±14	0.056543±37
ALL-49	49	0.41	3.057	105.61	0.0838±1	—	8.3802±14	0.70352±8	0.056481±21
ALL-56	84	0.58	0.424	143.60	0.00852±1	—	8.3653±18	0.69862±10	0.056589±27
ALL-68	78	0.81	1.045	170.87	0.01768±4	—	8.3764±13	0.69947±8	0.056461±21
ALL-70	242	1.21	0.410	158.84	0.00746±1	2.613	8.3785±23	0.69898±14	0.056541±35
Allende fine-grained Ca–Al inclusions									
ALL-5	161	0.13	15.45	22.219	2.028±7	—	8.3738±18	0.79511±12	0.056491±28
ALL-24	15	0.10	8.438	49.675	0.492±2	—	8.3680±23	0.72999±14	0.056557±35
ALL-30	20	0.08	8.581	44.424	0.560±2	—	8.3720±21	0.73449±13	0.056522±32
ALL-40	20	0.12	6.057	31.156	0.564±1	—	8.3740±24	0.73318±15	0.056472±36
ALL-72	53	0.07	4.241	20.641	0.596±1	—	8.3758±25	0.73586±15	0.056484±38
ALL-76 margin	29	0.21	5.644	57.979	0.2816±4	2.599	8.3660±16	0.71503±10	0.056589±25
ALL-76 core	103	0.12	6.587	30.287	0.629±1	—	8.3549±20	0.72362±13	0.056617±31
ALL-76 core	28	0.07	6.245	32.066	0.5635±7	2.613	8.3518±29	0.73368±18	0.056673±45

* Weight of sample dissolved in mg.

† The atomic ratio of sample Sr to spike Sr in the spiked analysis.

‡ Errors are 2 s.e.m.

§ Mantle nodules from South African kimberlite pipes: AJE 62, Mothae; BD 2344 and 2384, Bultfontein; JJG 846, Jagersfontein.

‖ Sources of meteorites: Parnallee, British Museum (Natural History), off BM34792; Richardton, Arizona State University, 100 h ASU; Barwell, British Museum (Natural History), off BM1966,59; Bruderheim, University of Alberta, B.93 UA; Allende, Smithsonian Institution.

but I feel that the generation of mass fractionation by diffusion seems unlikely. Vaporisation events, thought by many to be an important part of CAI history[25,26], are for the purposes of isotopic mass effects the reverse of condensation, and are thus also incapable of leaving an isotopically light solid from a normal starting material.

If the CAI formed from a vapour with isotopically light Sr, then they cannot have formed from the bulk of the solar nebula, which had overall 'normal' Sr, as shown here. They could have formed early in some restricted part of an inhomogeneous nebula[24,27], which had attained a light-isotope enriched Sr composition by: (1) prior condensation of materials which are isotopically heavy in Sr. These would have to be unsampled, and at least as refractory as CAI; or (2) vapour transport processes involving a kinetic isotope effect, such that one region of the vapour became enriched in light isotopes.

If the CAI did form in some restricted, fractionated region of the solar nebula, then the fact that the white outer part of the fine-grained CAI ALL-76 is closer to 'normal' Sr than the pink core may either indicate the beginning of equilibration of this anomalous region with the whole, or of the movement of that CAI as a solid object into a more normal region of the nebula. Alternatively, the white zone could merely represent equilibration of CAI with matrix during the isotopic disturbance demonstrated from $^{87}Sr/^{86}Sr$ data.

An entirely separate possibility is that the CAI are in part presolar objects[25,28], so that the Sr compositions are not *a priori* expected to be related to the remainder of the Solar System. Gas–dust fractionation in the presolar interstellar medium has been proposed to account for mass fractionation effects in CAI[29]. In this scheme, the refractory Ca–Al-rich dust and grains, which are isotopically heavy, become separated from the vapour, which is isotopically light, resulting in mass separation of the elements. For the Sr data, this scheme encounters the difficulty that the Ca–Al materials seem to be all isotopically light, inconsistent with their proposed role as isotopically heavy interstellar grains and dust. Note, however, that true type A CAI have not been sampled in this study. Although the bulk Sr of the environment where the sampled CAI formed could have been isotopically light ($^{88}Sr/^{86}Sr < 8.35$), it would then be remarkable that this presolar bulk Sr composition was related to the Solar System Sr composition along a mass fractionation line.

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Intrusion of basaltic sills into highly porous sediments, and resulting hydrothermal activity

Gerhardt Einsele[1], Joris M. Gieskes[2], Joseph Curray[2], David M. Moore[2], Eduardo Aguayo[3], Marie-Pierre Aubry[4], Daniel Fornari[5], José Guerrero[6], Miriam Kastner[2], Kerry Kelts[2], Mitchell Lyle[7], Yasumochi Matoba[8], Adolfo Molina-Cruz[9], Jeffrey Niemitz[10], Jaime Rueda[3], Andrew Saunders[11], Hans Schrader[7], Bernd Simoneit[12] & Victor Vacquier[2]

[1]Geologisches Institut, 74 Tübingen, FRG; [2]Scripps Institution of Oceanography, La Jolla, California 92093; [3]Instituto Mexicano del Petroleo, Mexico 14, D.F.; [4]Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543; [5]Lamont-Doherty Geological Observatory, Palisades, New York 10964; [6]Universidad Nacionál Autonoma de Mexico, Mexico 20, D.F.; [7]School of Oceanography, Oregon State University, Corvallis, Oregon 97331; [8]Department of Geology, Stanford University, Stanford, California 94305; [9]CICESE, Ensenada, Baja California, Mexico; [10]Department of Geology, Dickinson College, Carlisle, Pennsylvania 17013; [11]Department of Geological Sciences, Bedford College, Regent's Park, London NW1, UK; [12]Department of Geology, University of California, Los Angeles, California 90024

Sill intrusions into highly porous sediments in the Guaymas Basin, Gulf of California, lead to low-grade metamorphism, thermal alteration and migration of organic compounds, marked changes in interstitial water chemistry, and large-scale expulsion of heated pore fluids. The latter process creates space for the intruding magma and initiates a hydrothermal system, which can explain the observed hydrothermal deposits around fault scarps on the basin floor.

STUDIES of the genesis of several land-based ore deposits show that their formation is due to the intrusion of basaltic magma into deep water marine sediments[1,2]. Many geologists exploring such ore bodies believe that the sources of ore-forming elements are the sediments adjacent to the hydrothermal deposits[3,4]. Due to subsequent faulting, folding and metamorphism, however, the mechanisms of magmatic intrusions into soft sediments and their possible effects on the formation of hydrothermal deposits are not yet fully understood.

Hydrothermal deposits associated with mid-oceanic spreading centres, such as the Galapagos Rift hydrothermal system[5–7], occur generally in conditions where sedimentation rates are

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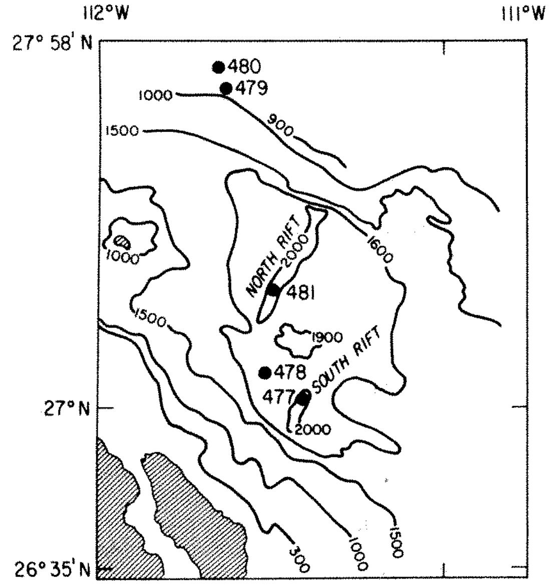

Fig. 1 Map of Guaymas Basin, depth contours in metres.

relatively slow and where magma extrudes onto the sea floor. There is strong evidence that seawater circulating through the basalts of Layer II is the convecting fluid in most mid-ocean ridge systems[5-8]. Hydrothermal waters are occasionally trapped in brine pools to form ore deposits, as has been discovered in the Red Sea[9].

At a time when studies of marine hydrothermal systems at ocean ridge crests are gaining momentum by the discovery of hydrothermal mounds and discharge vents[7], Leg 64 of the Deep Sea Drilling Project (DSDP) has drilled a hydrothermal system in the Guaymas Basin of the Gulf of California in a very different setting. In the Gulf of California, which represents a newly evolving ocean basin characterised by high sediment accumulation rates, oceanic basement is formed in a manner different from the more normally observed ophiolite, pillow basalt sequence of the later stages of ocean evolution.

Observations

The Guaymas Basin was formed during approximately the past 3.5 Myr by ocean floor spreading[10]. Two rift depressions (Fig. 1) lie in the otherwise flat floor of the basin, offset about 20 km by a transform fault. Both the north-east and south-west margins of the basin are the scarps of longer transform faults which connect to spreading axes in the next basins to the north and south. The entire basin and both rifts have been well explored by seismic and heat-flow measurements[11-13]. Three drilling sites were occupied in the basin during Leg 64—Sites 477, 478 and 481 (Fig. 1).

All three sites drilled thick sections of turbidites, composed of muddy diatomaceous oozes and plagioclase-rich terrigenous sands to muds[14]. Accumulation rates exceed 1,200 m Myr^{-1} on the basin floor and may be higher in the rift depressions. In four holes (477, 477A, 478 and 481A) basaltic to coarse-grained doleritic sills were encountered—several at shallow burial depths[14] (Fig. 2).

Site 477 in the southern rift consisted of two holes, offset by only 165 m. In Hole 477 the sill consists of altered dolerite, and in Hole 477A a less altered dolerite sill was encountered at shallower depths. Conductive heat flow was estimated from shipboard measurements to be at least 20 heat-flux units (HFU = 10^{-6} cal cm^{-2} s^{-1}), similar to that measured in the immediate vicinity by Lawver *et al.*[13]. Temperatures increase gradually with depth below the sills, thus indicating the presence of a major heat source below the drilled section of this site. Substantial increases with depth occur in dissolved chloride, lithium and manganese, especially below the sills (Fig. 3). Dolomite, anhydrite and pyrite occur in fine-grained, clay-rich sediments below the upper sills. Below 180 m the mineral paragenesis includes epidotes (4–10% Fe), quartz, albite and chlorite. These hydrothermal minerals indicate temperatures up to 300°C (ref. 15). Carbon/nitrogen ratios of organic matter increase rapidly with depth below 150 m and the organic matter is devoid of nitrogen below 200 m. The expulsion of all organic nitrogen also indicates very high temperatures.

Site 478 is located outside the present rift zones and therefore its sediments and basaltic intrusions are probably older. Heat flow is estimated at 3.65 HFU. Only minor increases in dissolved chloride and lithium occur, and dissolved manganese values are near the detection limits (<5 μM). Various changes have occurred in the sediments around the sills, including the remobilisation of silica, the formation of dolomite, and the recrystallisation of clay minerals. No higher temperature minerals have been observed.

Site 481 in the northern rift is characterised by a heat flow of about 4 HFU. Dissolved chloride and lithium concentrations (Fig. 3) clearly indicate the influence of the uppermost sill complex. No dissolved manganese was detected (<5 μM). Hydrocarbon (C_2–C_5) gas data show a thermal component above and below the sills, with a series of three maxima above the sill. These observations are similar to those in DSDP Site 368[16,17]. Carbon/nitrogen ratios of organic matter in Sites 478 and 481 do not change dramatically near the sills, indicating that sediments in the vicinity of the sills were not heated excessively. Oxygen isotopic analysis of recrystallised calcite, at a distance of about 1 m above the sill of Hole 481A, indicates a temperature of formation of ~150 °C.

Hydrothermal system

The following observations in the sediments above the doleritic intrusions are evidence of the sill-like nature of these intrusions: (1) decreases in porosities (Fig. 2); (2) disappearance of diatoms;

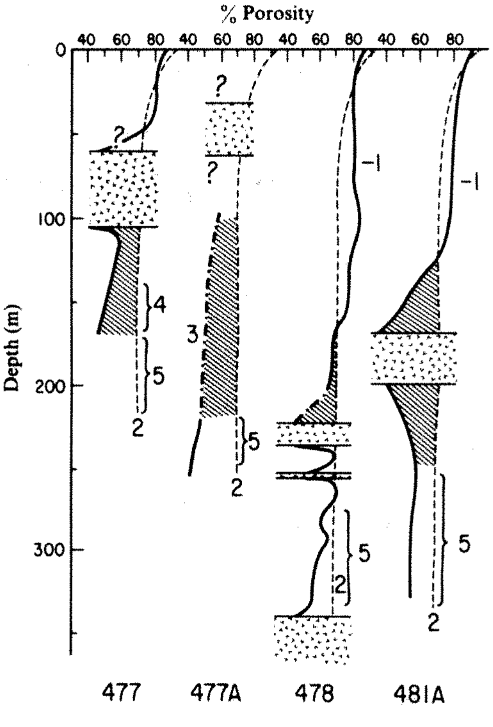

Fig. 2 Sill-induced reduction of porosity (hatched areas) in Guaymas Basin drill sites. 1, Shipboard porosity measurements; 2, shipboard porosity Site 479 (reference line); 3, porosity curve Hole 477A, deduced from density log; 4, porosity reduction due to deeper intrusions? 5, sections not considered for calculation of water loss (Table 1), though some effect may be caused by compaction due to upper sill load (in Site 478, definitely affected by lower sill).

Table 1 Reduction of porosity and water loss by intrusion of the uppermost sill (compare Fig. 2; for symbols and formulae, see text)

Hole	Thickness of sediment sections influenced by uppermost sill h_2	(m)	Loss of water (as height of water column) h_p (m) From single sections	Total	Thickness of uppermost sill (m)
477	Above sill	?13	7.3		
	Below sill	7	3.0	~44	45
		69*	33.4		
477A	Above sill	?	?		
	Below sill	35	?	At least 75	30
		120†	75.6		
478	Above sill	56	12·3	~14§	17
	Below sill	12	1.5		
481	Above sill	45	24.9	~53	30
	Below sill	50	28.6		

* Part of the porosity decrease of this section may be the result of a deeper hot intrusion. On the other hand, the sediments below those that were drilled are also affected by the load of the uppermost sill.

† Porosity deduced from density log. The cored section from 220 to 260 m with $n_2 \sim 45\%$ may be influenced by a deeper sill and is therefore not considered.

‡ Including thin sill at ~250 m depth. It is assumed that this sill has intruded simultaneously with the uppermost sill.

§ Minimum value, since real porosity reduction was probably larger than indicated by reference line (2) in Fig. 2.

(3) dolomite formation and calcite recrystallisation at elevated temperatures; (4) clay mineral recrystallisation and (5) organic matter decomposition.

The sharp decreases in porosity above and below the sill intrusions are particularly interesting. These decreases are clearly related to the effects of the intruding hot magmas, which cause the migration of pore waters away from the sills. The upper limits of the zone of reduced porosity presumably define the minimum thickness of sediment above the sills at the time of sill intrusion.

On intrusion of the sills, temperatures at the sill-sediment contact may rise to as high as 400 °C (the critical point for a pure 3·5% sodium chloride solution is 304 bar and 408 °C). In ridge-crest geothermal systems estimated temperatures at which seawater rock interactions occur are at least 300 °C[7]. Thus the pore waters in the immediate vicinity of the sills will rise to the boiling point. Most of the heat contained in the sill between 1,200 and 400 °C will be removed by the steam. Rough calculations indicate that for a sill of 30 m thickness, temperatures at the outer rims of the sill will drop to below the boiling point within a period of five years. From then on, heat will be lost from the sill by conduction through the sediments. Cooling of such a sill to 15 °C will be accomplished in ~50,000 yr. At sedimentary contacts in exposures on land, where original porosities may have been much lower, heating of material surrounding the intruding sills can be considerable, as evidenced by coal seams turned to cinder coal[19], fusion of sediments[20], and clastic dykes of liquified material intruded in the sills from the underlying sediments[21].

From the reduction in porosity in the contact zones, the amount of expelled pore water can be calculated in terms of the reduction in sediment thickness; this requires an estimate of the porosities before the occurrence of the sill intrusion. For Holes 477, 477A, 478 and 481A it has been assumed that the original porosities were similar to those in Site 479 (at the northern end of the Guaymas Basin, Fig. 1) at corresponding depths. These sediments have not been affected by intruding magma, and their composition resembles that in the basin sites. If there is a difference, the porosities in the basin sites were higher than in Site 479 because of their more turbiditic character and their faster sedimentation rates. Therefore, our calculations represent minimum values for the water losses.

The height h_p of expelled pore water can be found using the formula

$$h_p = h_1 - h_2 = h_2\left(\frac{100-n_2}{100-n_1}\right) - 1$$

where h_1, n_1 are the original height and porosity of a certain sediment section; and h_2, n_2 are the present (reduced) height, porosity of same sediment section.

It follows from the above formula that h_p is not a linear function of the reduction in porosity. If the porosity is reduced from 80 to 70%, $h_p = 0.5\ h_2$, that is, if $h_2 = 10$ m (present height of sediment section), 5 m of pore water has been lost and the original height of the sediment column was 15 m. If a decrease from 60 to 50% in porosity occurs, h_p is only $0{\cdot}2\ h_2$, and so on. Using an integrative model, the water loss from the contact zones above and below the uppermost, presumably youngest, sill was calculated (Table 1).

From the results of these calculations two principal conclusions can be drawn:

(1) At all sites the total water loss from the sediments caused by the uppermost sill is of the same order of magnitude (in height of sediment column) as the thickness of the sill. This suggests that the water removal from the neighbouring sediments creates space for the intruding magma and that a sill intrusion into highly porous sediments does not necessarily cause a large change in relief at the sea floor.

(2) If a sill of 30–40 m thickness can expel, on average, a water column of ~40 m (10 m above and 30 m below the sill), and if it occupies (perhaps together with neighbouring sills) an area of 1 km², ~40 × 10⁶ m³ of pore fluid are forced to the sea floor. This water migrates through somewhat tilted beds of relatively good permeability, for example, the sandy base of turbidites, until it reaches one of the many faults known from seismic records. These act as conduits leading the heated waters upwards (Fig. 5). At some later stage, some of the turbidites may become lithified and form cap rocks. But fissures within these layers or in the cooling sill create sufficient permeability for water to escape laterally. The sill also provides additional overburden pressure on the underlying sediments, causing compaction and thus further water expulsion. Sediments precompacted

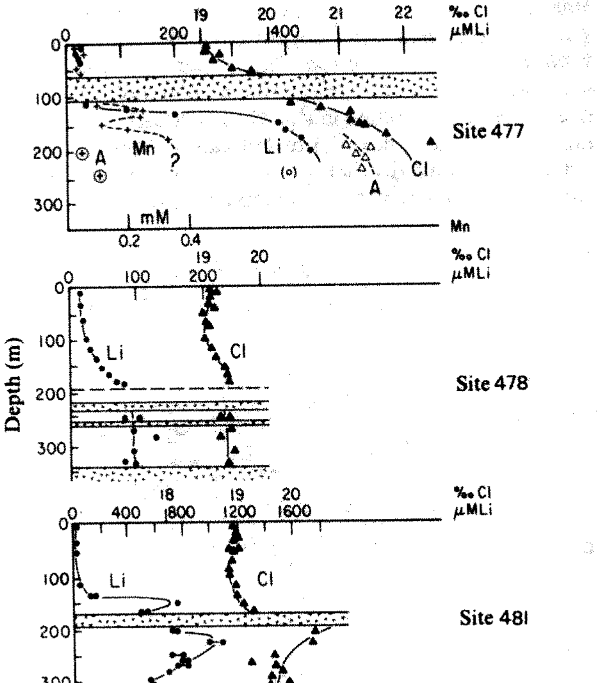

Fig. 3 Distribution of interstitial water lithium, chloride and manganese in Sites 477, 478 and 481.

by the intrusion of older, deeper sills probably are only slightly affected by the younger intrusions.

Preliminary data on the inorganic chemistry of the pore fluids of these sites indicate that with the expulsion of pore waters and the incomplete reaction of magnesium, substantial quantities of magnesium, silica, calcium and perhaps manganese, iron and phosphate, can be brought to the sea floor. This in turn can lead to the formation of hydrothermal deposits similar to those observed in the northern trough (talc, pyrrhotite, copper and zinc sulphide, manganese oxides)[22]. Preliminary calculations suggest that the emplacement of a 40 m thick sill could lead to the expulsion via the pore fluids of Ca, Mg and SiO_2 of the order of magnitude of $1–50\times10^6$ kg and $\sim0.01–0.1\times10^6$ kg of phosphate per km^2 of sill.

Geochemistry of hydrothermal sediments

Hydrothermal alteration of basalts at oceanic spreading centres leads to large increases in ^{3}He, Li, K and Ca in the hydrothermal fluids[7,23,24]. The data presented in Fig. 3 also indicate large increases in dissolved lithium. Although the source for this lithium may partly be due to hydrothermal alteration of the sediments, the enhanced ^{3}He levels observed in the bottom waters of the Guaymas Basin[25] indicate some magmatic contribution to the hydrothermal waters. Reversals in Li above and below the sill complex are probably related to the uptake of lithium into the sills from the pore fluids during present low-temperature alteration processes. Increases in dissolved chloride can be explained in terms of steam formation as well as by hydration of the sills.

The hydrothermal system of Site 477 is of particular interest. In this site, hydrothermal acitivity is associated with a deeper-seated heat source and not with the upper sills, which apparently have already cooled down considerably. Hydrothermal activity has probably been a long continuous process, leading to noticeable amounts of hydrothermal minerals, to increased metasomatism, and to the formation of high temperature phases such as epidote, albite, and chlorite. In Sites 478 and 481A, during and shortly after the intrusion of the igneous sills, heat flow must have been higher than at present. Most of these sills are considerably thinner than those observed in Holes 477 and 477A, so that cooling of these sills must have occurred at relatively rapid rates. This may explain why, in these circumstances, only moderate-temperature authigenic minerals (100–200 °C) have been detected, for example, dolomite, recrystallised calcite, recrystallised clay minerals. Also, only minor thermal decomposition of organic carbon has occurred in these sites. The three maxima in (C_3–C_5) hydrocarbon concentrations above the sill complex of Site 481 can be explained in terms of probable upward distillation (in three pulses) of C_3–C_5 hydrocarbons due to successive sill emplacements.

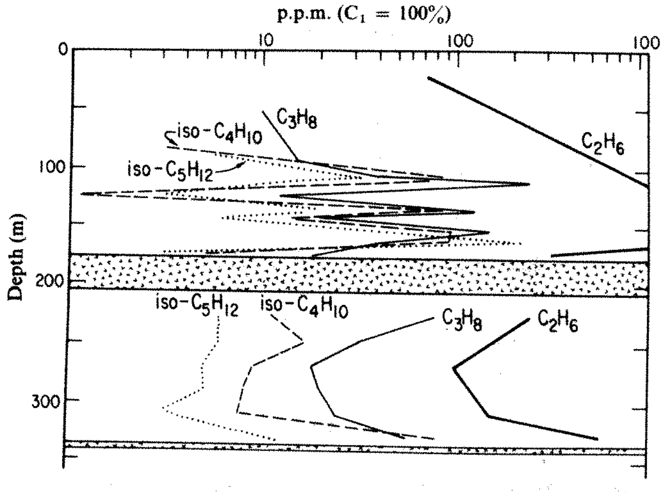

Fig. 4 Distribution of C_2–C_5 hydrocarbons, Site 481.

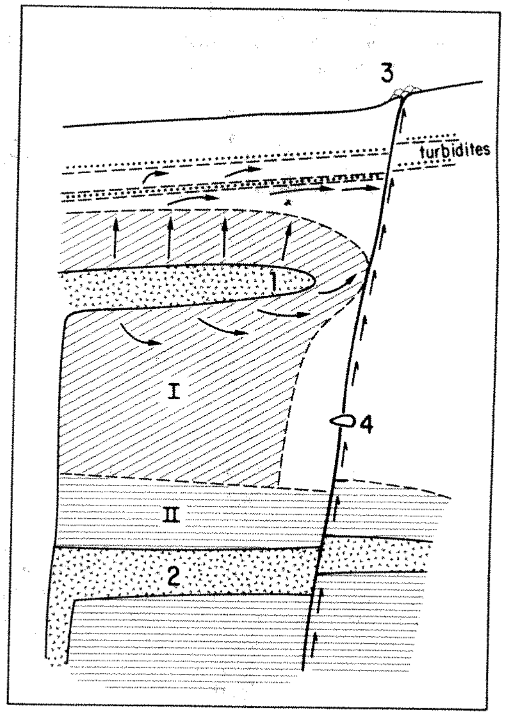

Fig. 5 Model of water expulsion due to porosity reduction as a result of intruding sills. 1, Uppermost sill; 2, older lower sill; 3, hydrothermal deposits around fault scarp; 4, Older, buried hydrothermal deposits. Area I, porosities affected by most recent sill intrusions; area II, porosities affected by older sill intrusions.

Conclusions

Drill sites in the Guaymas Basin in the Gulf of California demonstrate that during the initial stage of opening of some ocean basins, basaltic magma forming new oceanic basement intrudes as sills into rapidly deposited soft sediments in deep rift basins, thus creating a basement quite different from the normal ophiolite model concept[10]. Interaction between the hot sills and the surrounding sediments results in large changes in pore fluid composition (increases in dissolved lithium, chloride, manganese), in alteration of organic matter, in the formation of hydrothermal minerals, and in the large reduction in porosities in the sediment–sill contact zones. Large decreases in porosity imply substantial migration of heated pore fluids out of the sediments. This type of hydrothermal system is unlike those previously described on continents or at oceanic spreading centres. On the continent, hydrothermal systems are fed by recharge of meteoric water from neighbouring highlands, for example, the Salton Trough hydrothermal field[26]. At oceanic spreading centres without rapid accumulation of sediment, seawater circulates convectively through the basalts of Layer II. Our suggested mechanism does not require recharge of seawater into the sediments.

The dissolved constituents of the upward-migrating pore fluids deliver sufficient material for the precipitation of large amounts of magnesium silicates (as talc) and smaller quantities of iron and manganese compounds as oxides or sulphides, as well as minor amounts of phosphates and trace metals.

While the sills cool, the expulsion of pore waters slows down and finally ceases. The hydrothermal deposits formed at the sea floor are rapidly buried by younger sediments. New hydrothermal activity starts with the intrusion of younger sills into the overlying sections of unaltered sediments. Younger sills tend to develop on top of older ones because the magma can penetrate more easily into soft sediments than into sediments compacted by older sills.

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Coding potential and regulatory signals of the polyoma virus genome

Eiichi Soeda*, John R. Arrand, Nina Smolar, Jane E. Walsh & Beverly E. Griffin

Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, UK

The complete DNA sequence of the A2 strain of polyoma virus has been determined. It consists of 5,292 base pairs. The sequence is analysed in terms of its coding potential and sites of possible functional significance or structural interest. The polyoma virus genome is compared with those of related tumour viruses, simian virus 40 and BK virus.

POLYOMA virus is a small DNA tumour virus which was first isolated by Gross[1]. Polyoma virus grows in culture in a variety of mouse cell lines. It transforms other rodent cells (such as hamster or rat) in culture and can produce tumours in rodents. It is now considered reasonably certain that it codes for six proteins. These are the three 'early' proteins which have been designated as small, middle and large T antigens, and the three 'late' capsid proteins designated as (virus proteins) VP1, VP2 and VP3. Whether these are the only proteins encoded within the viral DNA and whether all the proteins are entirely virus coded is not known. Only a few distinct viral mRNA species have been identified, and, as yet, no early mRNA has been directly correlated with an early protein. The great interest in the 'early region', that is, the region transcribed before DNA replication occurs, follows from the fact that it is this region, or part of it, which seems to be responsible for the transformation *in vitro* of cells or for tumour formation *in vivo* (for general reviews see refs 2, 3).

Study of viral functions has been hampered by the fact that they are difficult to distinguish from those of the host cell. For example, infection with polyoma virus results not only in viral DNA replication, but also in the stimulation of cellular DNA replication. Thus, at the same time that viral functions begin to be expressed, many cellular functions are probably also switched on. There is also the problem, for instance, that virus proteins and messenger RNAs represent only a small proportion of the total proteins and mRNAs in a cell.

The DNA, on the other hand, is in relative terms, easier to study. The genome exists as a small, double-stranded, closed-circular molecule, the physical properties of which allow it to be isolated free from cellular DNA and characterised by the techniques of modern molecular biology. In sequencing polyoma virus DNA, we hoped to circumvent some of the problems mentioned above, estimate potential coding capacity of the virus, and assign parts of the genome to specific proteins. We also hoped to use the virus to elucidate control signals used in eukaryotic cells since it seemed reasonable to suppose that it might mimic its host.

An additional purpose in sequencing polyoma virus DNA was to compare the genome with that of closely related monkey and human viruses, simian virus (SV) 40 and BK virus. Much comment has been previously made of the similarities between the mouse (polyoma) and monkey (SV40) viruses. Although their hosts are different, the overall organisation of their DNAs has appeared to be remarkably similar[2]. This conclusion has been based primarily on the alignment of their respective messenger RNAs and their origins of replication relative to their DNAs, and on studies with a comparatively small number of mutants. However, attempts to hybridise the DNAs from the two viruses with each other in stringent conditions has revealed very little homology between the molecules.

The DNA sequence data, considered together with existing data on the viral proteins and mRNAs, now allow a more detailed description of the polyoma virus genome in functional terms. They also provide a predictive capacity not hitherto available for the virus. Moreover, a considerable homology with SV40 over about 80% of the genome is revealed, but large differences appear in the remaining 20%. These differences may be especially relevant since they appear to involve a transforming function[4] or regions which appear to have regulatory functions.

General features of polyoma virus DNA

The A2 (large plaque) strain of polyoma virus is composed of 5,292 base pairs. The DNA sequence is given in Fig. 1 and regions of special significance are listed in Table 1. The DNA contains 47.3% guanine–cytosine base pairs. An earlier estimate of this value, based on analysis of restriction endonuclease fragments, gave 47.0% (ref. 5). More than 90% of the viral DNA appears to be used in coding for proteins. The corollary to this is that somewhat less than 10% of the sequence may serve other functions, probably involved with control and regulation of viral expression. Data available at present suggest that a part of the sequence between the viral origin of replication and the initiation signal for early proteins may be dispensable[6], but there are no corresponding data about the sequence which lies between the origin of replication and the initiation signal for late

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*Permanent address Institute of Genetics, Misima 411, Japan.

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        10         20         30         40         50         60         70         80         90        100        110        120
                                              ▼           ▼
GGGGACCCCT GGCCCGCTTA CTCTGGAGAA AAAGAAGAGA GGCATTGTAG AGGCTTCCAG AGGCAACTTG TCAAAACAGG ACTGGCGCCT TGGAGGCGCT GTGGGGCCAC CCAAATTGAT
CCCCTGGGGA CCGGGCGAAT GAGACCTCTT TTTCTTCTCT CCGTAACATC TCCGAAGGTC TCCGTTGAAC AGTTTTGTCC TGACCGCGGA ACCTCCGCGA CACCCCGGTG GGTTTAACTA
3/5

       130        140        150        160        170        180        190        200        210        220        230        240
ATAATTAAGC CCCAACCGCC TCTTCCCGCC TCATTTCAGC CTCACCACCA TCATGGATAG AGTTCTGAGC AGAGCTGACA AAGAAAGGCT GCTAGAACTT CTAAAACTTC CCAGACAACT
TATTAATTCG GGGTTGGCGG AGAAGGGCGG AGTAAAGTCG GAGTGGTGGT AGTACCTATC TCAAGACTCG TCTCGACTGT TTCTTTCCGA CGATCTTGAA GATTTTGAAG GGTCTGTTGA

       250        260        270        280        290        300        310        320        330        340        350        360
ATGGGGGGAT TTTGGAAGAA TGCAGCAGGC ATATAAGCAG CAGTCACTGC TACTGCACCC AGACAAAGGT GGAAGCCATG CCTTAATGCA GGAATTGAAC AGTCTCTGGG GAACATTTAA
TACCCCCCTA AAACCTTCTT ACGTCGTCCG TATATTCGTC GTCAGTGACG ATGACGTGGG TCTGTTTCCA CCTTCGGTAC GGAATTACGT CCTTAACTTG TCAGAGACCC CTTGTAAATT

       370        380        390        400        410        420        430        440        450        460        470        480
AACTGAAGTA TACAATCTGA GAATGAATCT AGGAGGAACC GGCTTCCAGG TAAGAAGGCT ACATGCGGAT GGGTGGAATC TAAGTACCAA AGACACCTTT GGTGATAGAT ACTACCAGCG
TTGACTTCAT ATGTTAGACT CTTACTTAGA TCCTCCTTGG CCGAAGGTCC ATTCTTCCGA TGTACGCCTA CCCACCTTAG ATTCATGGTT TCTGTGGAAA CCACTATCTA TGATGGTCGC
                                          5/4

       490        500        510        520        530        540        550        560        570        580        590        600
GTTCTGCAGA ATGCCTCTTA CCTGCCTAGT AAATGTTAAA TACAGCTCAT GTAGTTGTAT ATTATGCCTG CTTAGAAAGC AACATAGAGA GCTCAAAGAC AAATGTGATG CCAGGTGCCT
CAAGACGTCT TACGGAGAAT GGACGGATCA TTTACAATTT ATGTCGAGTA CATCAACATA TAATACGGAC GAATCTTTCG TTGTATCTCT CGAGTTTCTG TTTACACTAC GGTCCACGGA

       610        620        630        640        650        660        670        680        690        700        710        720
AGTACTTGGA GAATGTTTTT GTCTTGAATG TTACATGCAA TGGTTTGGAA CACCAACCCG AGATGTGCTG AACCTGTATG CAGACTTCAT TGCAAGCATG CCTATAGACT GGCTGGACCT
TCATGAACCT CTTACAAAAA CAGAACTTAC AATGTACGTT ACCAAACCTT GTGGTTGGGC TCTACACGAC TTGGACATAC GTCTGAAGTA ACGTTCGTAC GGATATCTGA CCGACCTGGA

       730        740        750        760        770        780        790        800        810        820        830        840
GGATGTGCAC AGCGTGTATA ATCCAAGTAA GTATCAAGAG GGCGGGTGGG TATTTACGGC CTATATTCTT ACAGGGCTCT CCCCCTAGAA CGGCGGAGCG AGGAACTGAG GAGAGCGGCC
CCTACACGTG TCGCACATAT TAGGTTCATT CATAGTTCTC CCGCCCACCC ATAAATGCCG GATATAAGAA TGTCCCGAGA GGGGGATCTT GCCGCCTCGC TCCTTGACTC CTCTCGCCGG

       850        860        870        880        890        900        910        920        930        940        950        960
ACAGTCCACT ACACGATGAC TACTGGTCAT TCAGCTATGG AAGCAAGTAC TTCACAAGGG AATGGAATGA TTTCTTCAGA AAGTGGGACC CCAGCTACCA GTCGCCGCCT AAGACTGCCG
TGTCAGGTGA TGTGCTACTG ATGACCAGTA AGTCGATACC TTCGTTCATG AAGTGTTCCC TTACCTTACT AAAGAAGTCT TTCACCCTGG GGTCGATGGT CAGCGGCGGA TTCTGACGGC

       970        980        990       1000       1010       1020       1030       1040       1050       1060       1070       1080
AGTCTTCTGA GCAACCCGAC CTATTCTGTT ATGAGGAGCC ACTCCTATCC CCCAACCCGA GTTCTCCAAC AGATACACCC GCACATACTG CTGGAAGAAG ACGAAATCCT TGTGTTGCTG
TCAGAAGACT CGTTGGGCTG GATAAGACAA TACTCCTCGG TGAGGATAGG GGGTTGGGCT CAAGAGGTTG TCTATGTGGG CGTGTATGAC GACCTTCTTC TGCTTTAGGA ACACAACGAC

      1090       1100       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
AGCCCGATGA CAGCATATCC CCGGACCCCC CCAGAACTCC TGTATCCAGA AAGCGACCAA GACCAGCTGG AGCCACTGGA GGAGGAGGAG GAGGAGTACA TGCCAATGGA GGATCTGTAT
TCGGGCTACT GTCGTATAGG GGCCTGGGGG GGTCTTGAGG ACATAGGTCT TTCGCTGGTT CTGGTCGACC TCGGTGACCT CCTCCTCCTC CTCCTCATGT ACGGTTACCT CCTAGACATA
                        4/8

      1210       1220       1230       1240       1250       1260       1270       1280       1290       1300       1310       1320
TTGGACATCC TACCGGGGGA ACAAGTACCC CAGCTCATCC CCCCCCCTAT CATTCCCAGG GCGGGTCTGA GTCCATGGGA GGGTCTGATT CTTCGGGATT TGCAGAGGGC TCATTTCGAT
AACCTGTAGG ATGGCCCCCT TGTTCATGGG GTCGAGTAGG GGGGGGGATA GTAAGGGTCC CGCCCAGACT CAGGTACCCT CCCAGACTAA GAAGCCCTAA ACGTCTCCCG AGTAAAGCTA
             8/7

      1330       1340       1350       1360       1370       1380       1390       1400       1410       1420       1430       1440
CCGATCCTAG ATGCGAGTCA GAGAATGAGA GCTACTCACA GAGCTGCTCT CAGAGCTCAT TCAATGCAAC GCCACCTAAG AAGGCTAGGG AGGACCCTGC TCCTAGTGAC TTTCCTAGCA
GGCTAGGATC TACGCTCAGT CTCTTACTCT CGATGAGTGT CTCGACGAGA GTCTCGAGTA AGTTACGTTG CGGTGGATTC TTCCGATCCC TCCTGGGACG AGGATCACTG AAAGGATCGT

      1450       1460       1470       1480       1490       1500       1510       1520       1530       1540       1550       1560
GCCTTACTGG GTATTTGTCT CATGCTATTT ATTCTAATAA AACGTTCCCG GCATTTCTAG TATACTCCAC CAAAGAGAAA TGCAAACAAT TATATGATAC CATAGGGAAG TTCAGGCCCG
CGGAATGACC CATAAACAGA GTACGATAAA TAAGATTATT TTGCAAGGGC CGTAAAGATC ATATGAGGTG GTTTCTCTTT ACGTTTGTTA ATATACTATG GTATCCCTTC AAGTCCGGGC
                                                     7/2

      1570       1580       1590       1600       1610       1620       1630       1640       1650       1660       1670       1680
AATTCAAATG CCTGGTCCAT TATGAGGAGG GGGGCATGCT GTTCTTTCTA ACTATGACTA AGCACAGGGT TTCAGCAGTT AAGAATTATT GCTCTAAGCT TTGCCGCAGC TTCCTAATGT
TTAAGTTTAC GGACCAGGTA ATACTCCTCC CCCCGTACGA CAAGAAAGAT TGATACTGAT TCGTGTCCCA AAGTCGTCAA TTCTTAATAA CGAGATTCGA AACGGCGTCG AAGGATTACA

      1690       1700       1710       1720       1730       1740       1750       1760       1770       1780       1790       1800
GTAAGGCAGT CACCAAGCCT ATGGAATGCT ATCAAGTTGT AACCGCAGCA CCATTTCAGT TAATAACAGA AAATAAGCCA GGCCTCCACC AATTCGAGTT TACAGACGAG CCAGAAGAAC
CATTCCGTCA GTGGTTCGGA TACCTTACGA TAGTTCAACA TTGGCGTCGT GGTAAAGTCA ATTATTGTCT TTTATTCGGT CCGGAGGTGG TTAAGCTCAA ATGTCTGCTC GGTCTTCTTG

      1810       1820       1830       1840       1850       1860       1870       1880       1890       1900       1910       1920
AGAAAGCAGT AGACTGGATT ATGGTAGCAG ACTTTGCACT AGAAAACAAC CTTGATGATC CCCTGTTAAT TATGGGGTAT TATCTTGATT TTGCCAAAGA GGTTCCTTCA TGCATAAAGT
TCTTTCGTCA TCTGACCTAA TACCATCGTC TGAAACGTGA TCTTTTGTTG GAACTACTAG GGGACAATTA ATACCCCATA ATAGAACTAA AACGGTTTCT CCAAGGAAGT ACGTATTTCA

      1930       1940       1950       1960       1970       1980       1990       2000       2010       2020       2030       2040
GTAGCAAAGA GGAAACCCGC CTCCAAATAC ATTGGAAAAA CCATAGAAAG CATGCAGAGA ATGCAGACCT CTTCCTGAAT TGTAAAGCTC AAAAGACAAT CTGTCAGCAG GCAGCTGCGA
CATCGTTTCT CCTTTGGGCG GAGGTTTATG TAACCTTTTT GGTATCTTTC GTACGTCTCT TACGTCTGGA GAAGGACTTA ACATTTCGAG TTTTCTGTTA GACAGTCGTC CGTCGACGCT

      2050       2060       2070       2080       2090       2100       2110       2120       2130       2140       2150       2160
GTCTGGCATC CAGGAGACTG AAATTAGTAG AGTGTACCCG CAGCCAGCTA TTAAAGGAGA GATTGCAACA GTCTCTCCTC AGGCTAAAAG AACTTGGCTC CTCCGATGCT CTACTCTACC
CAGACCGTAG GTCCTCTGAC TTTAATCATC TCACATGGGC GTCGGTCGAT AATTTCCTCT CTAACGTTGT CAGAGAGGAG TCCGATTTTC TTGAACCGAG GAGGCTACGA GATGAGATGG

      2170       2180       2190       2200       2210       2220       2230       2240       2250       2260       2270       2280
TAGCAGGTGT CGCTTGGTAC CAGTGTCTTT TAGAGGACTT TCCTCAAACC CTGTTTAAGA TGCTTAAGCT GCTAACAGAA AATGTGCCAA AACGACGCAA CATACTTTTT AGAGGACCAG
ATCGTCCACA GCGAACCATG GTCACAGAAA ATCTCCTGAA AGGAGTTTGG GACAAATTCT ACGAATTCGA CGATTGTCTT TTACACGGTT TTGCTGCGTT GTATGAAAAA TCTCCTGGTC

      2290       2300       2310       2320       2330       2340       2350       2360       2370       2380       2390       2400
TTAATTCAGG AAAGACAGGC CTAGCAGCCG CGCTTATTAG CCTGTTAGGA GGCAAGTCTC TCAACATAAA TTGCCCTGCA GATAAACTTG CTTTTGAGCT TGGTGTGGCA CAGGACCAGT
AATTAAGTCC TTTCTGTCCG GATCGTCGGC GCGAATAATC GGACAATCCT CCGTTCAGAG AGTTGTATTT AACGGGACGT CTATTTGAAC GAAAACTCGA ACCACACCGT GTCCTGGTCA

      2410       2420       2430       2440       2450       2460       2470       2480       2490       2500       2510       2520
TTGTGGTCTG TTTTGAAGAT GTAAAGGGTC AAATAGCCTT GAACAAACAA CTGCAGCCAG GGATGGGAGT GGCTAATCTA GATAATCTCA GGACTACCTG GAATGGGAGT GTAAAGGTCA
AACACCAGAC AAAACTTCTA CATTTCCCAG TTTATCGGAA CTTGTTTGTT GACGTCGGTC CCTACCCTCA CCGATTAGAT CTATTAGAGT CCTGATGGAC CTTACCCTCA CATTTCCAGT

      2530       2540       2550       2560       2570       2580       2590       2600       2610       2620       2630       2640
ATCTAGAAAA AAAGCACAGC AACAAAAGGT CACAACTCTT TCCACCCTGT GTGTGTACAA TGAATGAATA TCTCCTACCA CAAACAGTAT GGGCCCGGTT TCACATGGTG TTGGATTTCA
TAGATCTTTT TTTCGTGTCG TTGTTTTCCA GTGTTGAGAA AGGTGGGACA CACACATGTT ACTTACTTAT AGAGGATGGT GTTTGTCATA CCCGGGCCAA AGTGTACCAC AACCTAAAGT
                                                                                                       2/6

      2650       2660       2670       2680       2690       2700       2710       2720       2730       2740       2750       2760
CCTGCAAACC CCATCTGGCC CAATCTCTTG AAAAGTGTGA ATTTTTGCAA AGGGAAAGAA TTATTCAGAG TGGAGATACC CTTGCCCTAT TACTCATATG GAATTTCACT TCAGATGTAT
GGACGTTTGG GGTAGACCGG GTTAGAGAAC TTTTCACACT TAAAAACGTT TCCCTTTCTT AATAAGTCTC ACCTCTATGG GAACGGGATA ATGAGTATAC CTTAAAGTGA AGTCTACATA

      2770       2780       2790       2800       2810       2820       2830       2840       2850       2860       2870       2880
TTGATCCTGA TATTCAGGGT CTTGTGAAGG AGGTTCGTGA CCAGTTTGCT AGTGAGTGCT CCTACAGTTT GTTTTGTGAT ATACTTTGTA ATGTGCAAGA AGGCGACGAC CCCTTGAAGG
AACTAGGACT ATAAGTCCCA GAACACTTCC TCCAAGCACT GGTCAAACGA TCACTCACGA GGATGTCAAA CAAAACACTA TATGAAACAT TACACGTTCT TCCGCTGCTG GGGAACTTCC

      2890       2900       2910       2920       2930       2940       2950       2960       2970       2980       2990       3000
ACATATGTGA TATAGCTGAA TACACAGTTT ATTGAATAAA CATTAATTTC CAGGAAATAC AGTCTTTGTT TTTCCAAAGC GGTCAACATA GCGCGTCATA TCAGGGTCCC CCGGTACAGG
TGTATACACT ATATCGACTT ATGTGTCAAA TAACTTATTT GTAATTAAAG GTCCTTTATG TCAGAAACAA AAAGGTTTCG CCAGTTGTAT CGCGCAGTAT AGTCCCAGGG GGCCATGTCC
                                                                                                                            6/1

      3010       3020       3030       3040       3050       3060       3070       3080       3090       3100       3110       3120
TTCAGTCCCA TCATACACTC TAACCTCCTC TACCTGGGTG TTCTCCCCTT CCATGGGTTG GCCCTGCACT TGGGGGAGCA TGTTGTTGAA AAGGGAACTT ATGAGGGAGG CCATGGGATA
AAGTCAGGGT AGTATGTGAG ATTGGAGGAG ATGGACCCAC AAGAGGGGAA GGTACCCAAC CGGGACGTGA ACCCCCTCGT ACAACAACTT TTCCCTTGAA TACTCCCTCC GGTACCCTAT

      3130       3140       3150       3160       3170       3180       3190       3200       3210       3220       3230       3240
GGGATTTTTG ACCCATCTTT TTCTCAGGGT GATTTTGAAA TATCTGGGAA AGCCCTTCTC CAGTGATGAC ACATAGTTTC TTGTAACTCT CCAGCCCATT ATATCTACAC AGGAGAGGTA
CCCTAAAAAC TGGGTAGAAA AAGAGTCCCA CTAAAACTTT ATAGACCCTT TCGGGAAGAG GTCACTACTG TGTATCAAAG AACATTGAGA GGTCGGGTAA TATAGATGTG TCCTCTCCAT

      3250       3260       3270       3280       3290       3300       3310       3320       3330       3340       3350       3360
TAGGCCCTCT CCTTTACAGA GGGGCCCAAC TCCATTTTCA TCTAGGAGCA CAGTTGTCAG GGTGTTTGTG AACTGCAGGA CGGGTGGAGC TGTTGTGCCT CCAGTGTAAT TGCCAAAGTA
ATCCGGGAGA GGAAATGTCT CCCCGGGTTG AGGTAAAAGT AGATCCTCGT GTCAACAGTC CCACAAACAC TTGACGTCCT GCCCACCTCG ACAACACGGA GGTCACATTA ACGGTTTCAT

      3370       3380       3390       3400       3410       3420       3430       3440       3450       3460       3470       3480
CCTTGTGTTC TCATTTTTTG CTGGATCTGG ATGCCAGATT TCAACTGGAT ACATTCCGTC CTTATCCAGC TTGGCCTTGC TAATTGGATT CAGGACTTGG TCTTTGTTGA CCATGTCCTT
GGAACACAAG AGTAAAAAAC GACCTAGACC TACGGTCTAA AGTTGACCTA TGTAAGGCAG GAATAGGTCG AACCGGAACG ATTAACCTAA GTCCTGAACC AGAAACAACT GGTACAGGAA

      3490       3500       3510       3520       3530       3540       3550       3560       3570       3580       3590       3600
CTTTGTGATT GTTTTGATTG TTACTACCCC TTCTTCCTTG TATTTTGTTC TGGCATCTGT CACAAGTCCC TGGAGGTCAA GCGGTTCCCC GCCACCAGCA AACACATGAT ATTGGCTGCC
GAAACACTAA CAAAACTAAC AATGATGGGG AAGAAGGAAC ATAAAACAAG ACCGTAGACA GTGTTCAGGG ACCTCCAGTT CGCCAAGGGG CGGTGGTCGT TTGTGTACTA TAACCGACGG

      3610       3620       3630       3640       3650       3660       3670       3680       3690       3700       3710       3720
TTCCACTGGA GTGGAATTTC CTTTGTGTTT ACTGAATCTG TGGGTTTTGT TGAACCCATG CACATCTAAC AGTGAGCCAG AGCCCACCAC CTCGGTTTTC ACTGAGACTG CCTCCCACAT
AAGGTGACCT CACCTTAAAG GAAACACAAA TGACTTAGAC ACCCAAAACA ACTTGGGTAC GTGTAGATTG TCACTCGGTC TCGGGTGGTG GAGCCAAAAG TGACTCTGAC GGAGGGTGTA

      3730       3740       3750       3760       3770       3780       3790       3800       3810       3820       3830       3840
TTGTAGGGTG TCACAGGTGA GGTCCTCATT GAGGCATGGG AAGCTGGACT TTGCCATACT CCATGTGGGA AGTGTATTAT TTCGGGGAAT CCACGTATCT GATGTAGCCA AATTAATCCC
AACATCCCAC AGTGTCCACT CCAGGAGTAA CTCCGTACCC TTCGACCTGA AACGGTATGA GGTACACCCT TCACATAATA AAGCCCCTTA GGTGCATAGA CTACATCGGT TTAATTAGGG
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Fig. 1

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      3850       3860       3870       3880       3890       3900       3910       3920       3930       3940       3950       3960
TCTGCTCCAA CCATAGTATT GCCCTCCCTC TGTTAGGCTT TCAGGGGTGG GTGGCTGCCC CATTCTGGGG TTCAGAAAAG CTTCTATTTC TGTCACACTG TCTGGCCCTG TCACAAGGTC
AGACGAGGTT GGTATCATAA CGGGAGGGAG ACAATCCGAA AGTCCCCACC CACCGACGGG GTAAGACCCC AAGTCTTTTC GAAGATAAAG ACAGTGTGAC AGACCGGGAC AGTGTTCCAG

      3970       3980       3990       4000       4010       4020       4030       4040       4050       4060       4070       4080
CAGCACCTCC ATACCCCCTT TAATAAGCAG TTTGGGAACG GGTGCGGGTC TTGGACAGGC CTTTGTACAT TTTGTCTCGC ATTTAGAGAC GCCGCTTTTT CTTTTGGGGG CCATCTTCCT
GTCGTGGAGG TATGGGGGAA ATTATTCGTC AAACCCTTGC CCACGCCCAG AACCTGTCCG GAAACATGTA AAACAGAGCG TAAATCTCTG CGGCGAAAAA GAAAACCCCC GGTAGAAGGA

      4090       4100       4110       4120       4130       4140       4150       4160       4170       4180       4190       4200
CTATGACTGT TGCCCAAGTA GGTGTGATAT CACCGTACAG CCCTAGAATT AAAGGAAGCA TCCAGTCAGG AGTGACTCTT TGGTGGGACA CCACCTGGGC CTGATAGAAC TTTATCACCT
GATACTGACA ACGGGTTCAT CCACACTATA GTGGCATGTC GGGATCTTAA TTTCCTTCGT AGGTCAGTCC TCACTGAGAA ACCACCCTGT GGTGGACCCG GACTATCTTG AAATAGTGGA

      4210       4220       4230       4240       4250       4260       4270       4280       4290       4300       4310       4320
CACCTGACTC ATCCTGTATA TGTGCTGCAG GGGTAGGCCC ACCATTTCCC ATGCTCCCTT CTATGCGATT AAAGAGTGCT CTCCTCTGTG GTGGATTAAG ACCTAGTTGC CTATAATAGT
GTGGACTGAG TAGGACATAT ACACGACGTC CCCATCCGGG TGGTAAAGGG TACGAGGGAA GATACGCTAA TTTCTCACGA GAGGAGACAC CACCTAATTC TGGATCAACG GATATTATCA

      4330       4340       4350       4360       4370       4380       4390       4400       4410       4420       4430       4440
CACTTAGTGA TGAGTACCCT GAGCTCGCTG ATGAGGCACC TCTGTTGATT GCATCTATGG CTGACTGAGG AGCATTAGAA ACCACCCACC GGGTGTTTTC AAGTAGCCTA GCTAGGCCAT
GTGAATCACT ACTCATGGGA CTCGAGCGAC TACTCCGTGG AGACAACTAA CGTAGATACC GACTGACTCC TCGTAATCTT TGGTGGGTGG CCCACAAAAG TTCATCGGAT CGATCCGGTA
                                                                                                   4/3
      4450       4460       4470       4480       4490       4500       4510       4520       4530       4540       4550       4560
CCAGGAATGT ATGTGTCTGT CTTACAGTTA GTTCTCTTAC AGCTCCTTCC AGTCTGTGTT GTGTTTCCTG CACAACCATT TGCCACACAT ATCTTCCCAC GGAATGAAGT AGGCCATGGC
GGTCCTTACA TACACAGACA GAATGTCAAT CAAGAGAATG TCGAGGAAGG TCAGACACAA CACAAAGGAC GTGTTGGTAA ACGGTGTGTA TAGAAGGGTG CCTTACTTCA TCCGGTACCG

      4570       4580       4590       4600       4610       4620       4630       4640       4650       4660       4670       4680
CCCAATCATG TACTACATTT AGAGCATGAG CAAACTGATT AACTCCTGGG AAGTATATAT CGAGAAGGGC TGGATCCCGC CATGGTATCA ACGCCATATT TCTATTTACA GTAGGGACCT
GGGTTAGTAC ATGATGTAAA TCTCGTACTC GTTTGACTAA TTGAGGACCC TTCATATATA GCTCTTCCCG ACCTAGGGCG GTACCATAGT TGCGGTATAA AGATAAATGT CATCCCTGGA

      4690       4700       4710       4720       4730       4740       4750       4760       4770       4780       4790       4800
CTTCGTTGTG TAGGTACCGC TGTATTCCTA GGGAAATAGT AGAGGCACCT TGAACTGTCT GCATCAGCCA TATAGCCCCC GCTGTTCGAC TTACAAACAC AGGCACAGTA CTGACAAACC
GAAGCAACAC ATCCATGGCG ACATAAGGAT CCCTTTATCA TCTCCGTGGA ACTTGACAGA CGTAGTCGGT ATATCGGGGG CGACAAGCTG AATGTTTGTG TCCGTGTCAT GACTGTTTGG

      4810       4820       4830       4840       4850       4860       4870       4880       4890       4900       4910       4920
CATACACCTC CTCTGAAATA CCCATAGTTG CTAGGGCTGT CTCCGAACTC ATTACACCCT CCAAAGTCAG AGCTGTAATT TCGCCATCAA GGGCAGCGAG GGCTTCTCCA GATAAAATAG
GTATGTGGAG GAGACTTTAT GGGTATCAAC GATCCCGACA GAGGCTTGAG TAATGTGGGA GGTTTCAGTC TCGACATTAA AGCGGTAGTT CCCGTCGCTC CCGAAGAGGT CTATTTTATC

      4930       4940       4950       4960       4970       4980       4990       5000       5010       5020       5030       5040
CTTCTGCCGA GAGTCCCGTA AGGGTAGACA CTTCAGCTAA TCCCTCGATG AGGTCTACTA GAATAGTCAG TGCGGCTCCC ATTTTGAAAA TTCACTTACT TGATCAGCTT CAGAAGATGG
GAAGACGGCT CTCAGGGCAT TCCCATCTGT GAAGTCGATT AGGGAGCTAC TCCAGATGAT CTTATCAGTC ACGCCGAGGG TAAAACTTTT AAGTGAATGA ACTAGTCGAA GTCTTCTACC

      5050       5060       5070       5080       5090       5100       5110       5120       5130       5140       5150       5160
CGGAGGGCCT CCAACACAGT AATTTTCCTC CCGACTCTTA AAATAGAAAA TGTCAAGTCA GTTAAGCAGG AAGTGGACTA ACTGACGCAG CTGGCCGTGC GACATCCTCT TTTAATTAGT
GCCTCCCGGA GGTTGTGTCA TTAAAAGGAG GGCTGAGAAT TTTATCTTTT ACAGTTCAGT CAATTCGTCC TTCACCTGAT TGACTGCGTC GACCGGCACG CTGTAGGAGA AAATTAATCA

      5170       5180       5190       5200       5210       5220       5230       5240       5250       5260       5270       5280
TGCTAGGCAA CGCCCTCCAG AGGGCGTGTG GTTTTGCAAG AGGAAGCAAA AGCCTCTCCA CCCAGGCCTA GAATGTTTCC ACCCAATCAT TACTATGACA ACAGCTGTTT TTTTTAGTAT
ACGATCCGTT GCGGGAGGTC TCCCGCACAC CAAAACGTTC TCCTTCGTTT TCGGAGAGGT GGGTCCGGAT CTTACAAAGG TGGGTTAGTA ATGATACTGT TGTCGACAAA AAAAATCATA

      5290          8         18         28         38         48         58         68         78         88         98        108
TAAGCAGAGG CC
ATTCGTCTCC GG
              3/5
```

Fig. 1 The total sequence of polyoma virus DNA (A2 strain ref. 45), which contains 5,292 base pairs. The top strand contains a sequence with the same polarity as early mRNAs and the bottom strand contains a sequence with the same polarity as late mRNAs (ref. 46). The location of the eight *Hpa*II restriction endonuclease sites are indicated (for example, 3/5, 5/4). Using the convention previously adopted[11], the centre of the *Hpa*II 3/5 junction (CC↓GG) has been designated zero and nucleotides are numbered proceeding in a clockwise direction on the physical map (see Fig. 3). This *Hpa*II site probably includes the viral origin of replication, and such numbering allows easy comparison with the SV40 sequence of Fiers *et al.*[7], or the sequence as renumbered by Buchman *et al.*[47]. The location of the 11-base pair deletion found in the A3 strain of the virus[45], between nucleotides 43 and 55 (ref. 11), is indicated on the sequence by arrows (▼). The probable ATG initiation codon for the early proteins (position 173) and for the late proteins VP1(4074), VP3 (4657) and VP2 (5002) are shaded. Other sites of known or postulated significance are given in Table 1 and Fig. 3. Most of the sequence was derived using either the chemical sequencing procedure of Maxam and Gilbert[48], or the dideoxy method of Sanger *et al.*[49]. Over parts of the genome, both methods were used. Moreover, for most of the genome, both strands were sequenced. Details are given elsewhere[11,12,26,27]. Over 95% of the sequence was derived from studies on a single preparation of polyoma virus DNA, using a stock of A2 strain virus made from an isolate which was twice plaque purified.

proteins. With polyoma virus, for example, there are no extensive sequence duplications such as are found in the late region of SV40 (refs. 7, 8). For further consideration, the DNA is divided into 'coding' and 'noncoding' regions, and specific features are discussed in the relevant sections.

The coding regions

The early coding region extends from about 74.0 to 25.5 units clockwise on the physical map of polyoma virus DNA (ref. 9) and consists of about 2,700 base pairs. Assuming a co-linear relationship between DNA, RNA and protein, this amount of DNA would contain the coding capacity for proteins with a total molecular weight (MW) of about 100,000, and any splicing of the viral RNA molecules would reduce the estimate. However, the three known early proteins of polyoma virus, the small t, middle T and large T antigens, have been assigned combined MWs of about 175,000 (see ref. 10 and Table 2). The T antigens appear to be genuine virally coded proteins, so it is obvious that the virus needs extra coding sequences. From the DNA sequence, this appears to be accomplished by using more than one coding frame in a particular region of the DNA, that is, proteins are encoded in overlapping genes[11]. The coding potential of the early region is illustrated schematically in Fig. 2*a*. If the DNA is divided into its three coding frames and a solid block is placed within a frame whenever one of the triplet codons TAA, TAG or TGA (the putative termination signals) appear in the sequence, it is apparent that between 74 and 85 map units there is only one open coding frame. This has been designated frame 1. This frame is then closed, and between about 85 and 99 map units the other two frames (frames 2 and 3) are open. From 99 map units to the end of the early region, only one extensive frame (frame 2) is open, although short open areas are found in the other two frames. Thus, coding throughout the early region requires frameshifts[12,13]. These data are summarised in Fig. 2*a*, and protein assignments allowed by these and other data[14] are illustrated in Fig. 2*b*. From other studies of the proteins themselves, studies on mutants, and by analogy with SV40, frame 1 is believed to code initially for all three of the early proteins and they are thought to share common N-termini[15–17]. The bodies and C-termini of middle T antigen and large T antigen are encoded in frames 3 and 2, respectively. The C-terminus of small t antigen may lie in frame 1. Additional data taken from the DNA sequence (Fig. 1) which predict the location of the termination codons for each protein, postulate splices which occur in the mRNAs to produce the relevant proteins, and suggest the location of other significant or novel sites on the DNA are summarised in Table 1*a*. Some of these data have been published elsewhere[11–13].

One interesting aspect of the DNA sequence is that there is a strong bias in the distribution of termination codons. Whereas the termination signals TAA, TAG and TGA appear with about equal frequency through most of the early region in frames which are probably not being used for coding, this is not so for the region in frame 1 between about 86 and 95.5 map units. In this region, the only termination codon found in the DNA sequence is TGA. Were an occasional 'readthrough' of this signal to occur, a protein with MW of about 40,000 could be made entirely from frame 1, allowing, that is, for a suitable splice in the mRNA to remove the termination codon triplet (TAG) encoded in the DNA in this frame at position 806 (85.7 map units). This point is made not only because of this unusual

Table 1 Landmarks on polyoma virus (A2 strain) as suggested by DNA sequence

a, Early region (from about 1 to 2,920 nucleotides, see top strand, Fig. 1)

Nucleotide no.	Sequence	Significance	Refs
5263–45 (5267–18)	See Fig. 1	Region around viral origin of replication (hairpin loop)	11, 42, 52
87–97	GCCTTGGAGGC	Single *Bgl*I site (72.2 map units)	11, 52
5078–5085 5151–5158 120–127 272–279	TTAAAATA TTTAATTA TATAATTA TATAAGCA	'Hogness boxes'—potential early promoters ≥ 75% homology with canonical TATAAATA)	
54–58 142–146	CTTCC CCTTCC	Possible ribosomal binding sites	11, 53
173–175	ATG	Probable initiation codon for known early proteins (T antigens)	11, 16
512–698	See Fig. 1	Deletion in hr-t mutant, NG-18	54, 55
406–412 790–795	CCAG/GTA TACAG/G	Possible splicing signals for large T antigen messenger	12, 14
743–749 790–795	CCAA/GTA TACAG/G	Possible splicing signals for small t antigen messenger	12, 14
Either 762–767 790–795 Or 743–749 804–809	 GCGG/GTG TACAG/G CCAA/GTA CCTAG/A	Possible splicing signals for middle T antigen messenger	12, 14
705–808	See Fig. 1	Minimum region containing at least one termination codon in each coding frame	11
806–808	TAG	Proposed termination codon for small t antigen	12, 14
1,158–1,176	$(GGA)_6G$	Unusual triplet sequence repeat	11
1,215–1,219	$G\overset{\swarrow}{G}GGG$	Possible location($\swarrow$) of 5-MeC (on complementary strand)	11
1,476–1,481	AATAAA	Possible processing signal for unknown early mRNA	11, 14
1,498–1,500	TAG	Proposed termination codon for middle T antigen	11
1,560–1,565	GAATTC	Single *Eco*RI site (0/100 map units)	5, 11
2,222–2,776	See Fig. 1	Region of very high homology with either SV40 DNA or its predicted T antigen sequences	12, 13
2,584–2,785	See Fig. 1	Long open reading frame beginning with ATG. Could code for a small protein	12
2,913–2,915	TGA	Proposed termination codon for large T antigen	12
2,915–2,920	AATAAA	Possible processing signal for early mRNA(s)	12, 14

b, Late region (from about 5,292 to 2,908 nucleotides, see bottom strand, Fig. 1)

Nucleotide no.	Sequence	Significance	Refs
5,283–5,268	$TTAATACTA_8$	A, T-rich region near viral origin of replication	11
5,155–5,148	TTAAAAGA	'Hogness box'—potential late promoter	26
5,094–5,086	GACATTTTC	Possible location of 5′-ends of late mRNAs	32
5,023–5,018 5,081–5,076	TCAA/GTA TTAAG/A	Possible 'leader to leader' splice which gives rise to tandem repeats in late mRNAs	26, 31
5,091–5,078 5,066–5,060 5,012–5,001	AT_4CTAT_4AA AAAATTA AAT_4CAAA	AT-rich regions in noncoding sequence	26
5,037–5,031	TCTTCTG	Possible ribosomal binding site	26, 53
5,021–5,026	TGATCA	Single *Bcl*I site (65.4 map units)	
5,023–5,018 5,019–5,014	TCAA/GTA GTAAG/T	Possible 'leader to body' splice for 19S late mRNA (alternative, no splice)	26, 3
5,023–5,018 4,712–4,705	TCAA/GTA CCTAG/G	Possible splice for 18S late mRNA	26, 32
5,023–5,018 4,127–4,122	TCAA/GTA TCTAG/G	Possible splice for 16S late mRNA	27
5,002–5,000 4,657–4,655 4,074–4,072	ATG ATG ATG	Initiation codon for VP2 Initiation codon for VP3 Initiation codon for VP1	25–27
4,632–4,637	GGATCC	Single *Bam*I site (58.0 map units)	
4.045–4,043	TAA	Termination codon for VP2/VP3	26
4,074–4,046	See Fig. 1	Overlap region between genes coding for VP2/VP3 and VP1	26, 27
2,925–2,923	TAA	Termination codon for VP1	27
2,913–2,908	AATAAA	Possible processing signal for late mRNAs	12, 27

feature of the sequence, but also because a protein of MW 39,000, although not yet completely characterised, is frequently found together with the other polyoma virus early proteins[15]. Similarly, it is interesting to speculate that a second large T antigen observed on polyacrylamide gels, which has an MW slightly greater than usually found[18], could be due to read through of the UGA termination codon for large T antigen. Precedence for this suggestion appears in studies on the phage Qβ where a low level of readthrough of the UGA codon for the coat protein occurs to produce a longer, functional protein[19]. An alternative, and attractive, explanation is that TGA may not always specify a termination codon. In two mitochondrial systems, it has recently been suggested that UGA, instead of functioning as a termination codon, may code for tryptophan[20,21]. If either readthrough of UGA occurred or in polyoma virus UGA were a coding triplet, it is clear that this virus could specify more proteins than those which are known at present.

The late coding region extends from about 65 to 25.5 units anticlockwise on the physical map of polyoma virus DNA and consists of about 2,100 base pairs. This is enough information to code for proteins of approximate total MWs 77,000 in a co-linear fashion. The three known late proteins (VP1, VP2 and VP3) of polyoma virus have been assigned combined MWs of about 105,000 (see Table 2). However, it would appear that although they seem to be derived from independent mRNAs (ref. 22), all the amino acid sequences present in VP3 are also present in VP2. Therefore, the late proteins essentially require coding information for an MW of only about 80,000, which roughly corresponds to the prediction from the DNA sequence.

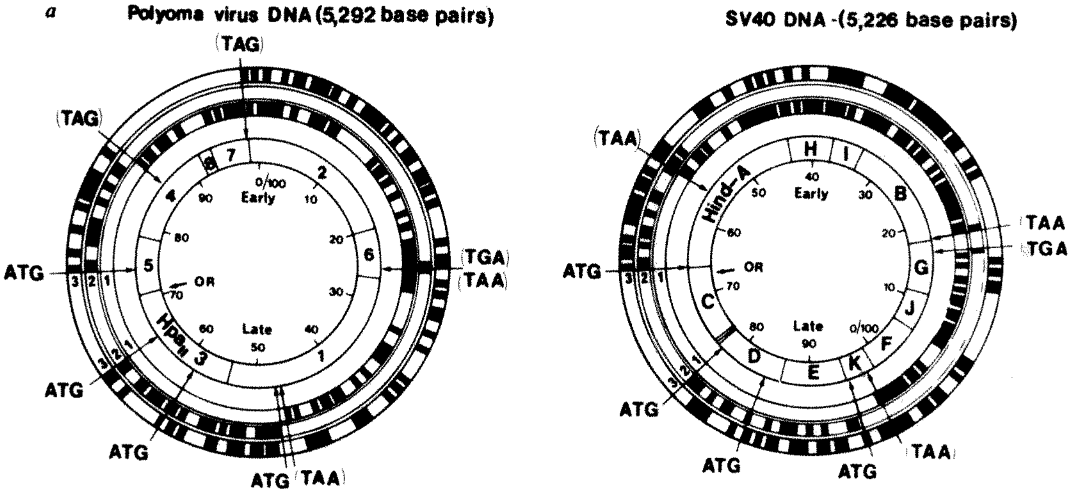
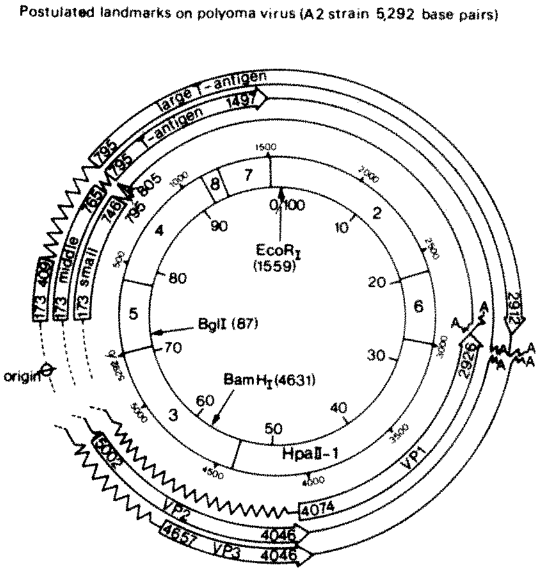


Fig. 2 *a*, A schematic diagram which divides the DNAs of polyoma virus and SV40 into three coding frames relative to their respective conventional physical maps. For polyoma virus, there are eight *Hpa*II fragments and the map is divided into 100 units with the *Eco*RI site at 0/100 map units[5]; for SV40, there are 13 *Hin*dII and *Hin*dIII fragments[7] and the map has been similarly divided with the *Eco*RI site at 0/100 map units. The conventional SV40 map (ref. 50) has been reorientated to allow a direct comparison with polyoma virus to be made. In each case, the viral origin of replication (OR) is also indicated. Whenever a termination codon (TAA, TAG, TGA) appears in the sequence within nine triplet codons (or 27 nucleotides) on the appropriate coding strand of the virus this is indicated by a block (■). For each virus within the early coding region, frame 1 begins at the A of the ATG codon which corresponds to the initiation codon (AUG) for the early proteins; consequently, coding frame 2 begins at the T and frame 3 at the G. By analogy, in the late region, frame 1 begins at the A of the ATG which specifies VP2, frame 2 at T and frame 3 at G. Since the early products of polyoma virus are transcribed off one strand of the DNA and late products off the other[51], early coding frames do not correspond to late coding frames. For polyoma virus, starting at the origin of replication and proceeding clockwise on the outside of the circle, are shown, in order, the single ATG initiation codon for the early proteins, and the TAG, TGA and TGA termination codons of small t, middle T and large T antigens, respectively. Proceeding anticlockwise (and from the opposite strand) the ATG initiation codons for VP2, VP3 and VP1 respectively, and the TAA termination codons for VP2/VP3 and VP1, respectively are shown. The diagram for SV40 corresponds in organisation to that of polyoma virus except that there are no signals for a middle T antigen. *b*, Landmarks on polyoma virus. The conventional *Hpa*II physical map is shown[5]. It has been divided into 100 units with the single *Eco*RI site at 0/100 map units. All other numbers refer to nucleotide numbers in Fig. 1. The postulated location on the genome of the six known virally coded proteins (the three early proteins, small t, middle T and large T antigens, and the late capsid proteins, VP1, VP2 and VP3) are indicated in the appropriate curved bars (see Table 1). Location of parts of messenger RNAs not coding for proteins are indicated as single, curved lines. DNA sequences absent in mRNAs, which have probably been removed by splicing, are indicated by jagged lines (ᨓ). Data for this diagram are found in Table 1. The coding region for the C-termini of small t antigen is assigned to the sequence between nucleotides 795 and 805, since this allows a protein with a size appropriate to this antigen[10] to be specified. Note that bodies of middle T and large T antigens are encoded in two differerent frames as are VP2/VP3 and VP1. There is an alternative splice for middle T antigen at a position between 747 and 800 map units; S_1 nuclease mapping data suggest that this is probably the correct one[14]. The coding frames used for each protein are shown in *a*.

b



The sequences of the N-termini of the three late proteins have been determined[25]. It is thus possible to position them accurately on the DNA sequence. As above, the coding potential of the late region sequence is illustrated schematically in Fig. 2*a*. It can easily be seen that proceeding anticlockwise on the physical map from the origin of replication, only one reading frame (designated frame 1), between about 65 and 47 map units, contains no termination codons. This frame is then closed, and between about 46.3 to 25.5 map units only frame 2 is open for any extensive region. Thus, coding over the entire late region requires two reading frames. There are other unblocked regions within each frame over the entire late region which could be used for coding small polypeptides although no proteins other than the three mentioned above are known. The protein assignments on the genome are shown in Fig. 2*b* and the data are summarised in Tables 1*b* and 2. In coding terms the late region generally contains no surprising features and an analysis of it has been given elsewhere[26,27]. As in SV40 (refs 7 and 8), the C-termini of VP2/VP3 overlap the N-terminus of VP1 although the overlap in polyoma virus is less extensive than that predicted for the former (see Fig. 2).

The codons used by polyoma virus are given in Table 3. What is immediately apparent is the small use made of any codon which incorporates CG. Although CGN and NCG are occasionally used, their use is well below average, whereas CG when it appears between codons (NNC–GNN) occurs with average frequency (data not shown). This result is consistent with earlier studies on the dinucleotide composition of polyoma virus DNA[28]. A surprising feature is the low use of the codon AUC for isoleucine or GUC for valine, and to a lesser extent CUC for leucine, although the same discrimination against NUC codons is observed with SV40 (refs 7 and 8).

In summary, polyoma virus contains more coding information than a co-linear relationship between DNA, RNA and proteins would suggest. In several parts of the genome, notably in about half of the early region, more than one coding frame is used. The DNA sequence allows data from studies on viral mutants, mRNAs and proteins to be correlated. It should be emphasised that the latter data are by no means extensive and it is possible that our ideas about the coding potential of polyoma virus will later appear too restricted. The sequence seems to imply this. As a by-product of these studies, which allow the DNA sequence to be aligned with the (known) polyoma virus proteins, we should now be able to construct mutants to test the current model for the functional viral genome, as illustrated in Fig. 2*b*.

The non-coding or control regions

There appear to be two noncoding regions in the polyoma virus genome interspersed between coding regions. One lies entirely within the early region, probably between nucleotides 746 and 795 (Fig. 1), and the other lies around, and includes, the viral origin of replication. The presumption with the former is that it contains the information which regulates splicing events so that they occur in such a way as to produce functional messengers for the three early proteins, the small t, middle T and large T antigens. The dinucleotides GT and AG are thought to be important splicing signals[29] and the coding strand of this region, as well as the sequence which immediately precedes it, has a number of these dinucleotides. Between positions 724 and 794 there are seven GT and five AG dinucleotide sequences. Note that the entire region under consideration can adopt a moderately stable secondary structure which resembles a clover-leaf (Fig. 3*a*). The hypothesis that a GT dinucleotide lies at the 5′-side of an intervening sequence and an AG lies at the 3′-side seems now to be supported by data from a wide variety of sources (for review, see ref. 30). The assumption can therefore be made that any one, or all of these dinucleotides, could theoretically be used in the recognition events that lead to RNA splicing, but it is not known if structures such as that shown in Fig. 3*a* are important. Although the preferred splicing sites for the three proteins, as deduced from S_1 single-strand specific nuclease studies[14], are given in Table 1*a*, it can be envisaged that through either error or some subsequent selection procedure, other sites might be used during the growth cycle of the virus. These could lead to a variety of messengers being produced which specify proteins with only slightly modified sequences[12]. At the level of sophistication being used to study the viral proteins, this would not be noticed. Nonetheless, small changes in amino acid sequence at a crucial site in the protein could easily alter the tertiary structure and thereby the function of a protein. By such a route, a small virus could greatly extend its coding capacity. Whether this is the case with polyoma virus, or whether, because of other structural factors the virus uses only a minority of its potential splicing signals, is not known.

The second noncoding region must contain control signals for viral replication and for the initiation of transcription. It should also contain information for the correct splicing events which produce functional late mRNAs[31,32]. This region extends from positions 172 downstream along the sequence given in Fig. 1 to, and including, position 5,003. Some of the nucleotides around the two *Hha*I restriction endonuclease sites at positions 85 and 96 (about 72 map units) are not essential to the virus[6]. Both these sites and the regions around them can be deleted, and the resulting mutants appear both to replicate and to transform cells with a facility which approximates that of the wild-type virus. Without further direct evidence, it may be assumed that the rest of the sequence in this noncoding region serves some function. A number of sites of possible relevance to control and regulation of the virus are pointed out in Table 1. One of the most important is, of course, the viral origin of replication. It has been suggested that the origin probably lies around, and may include, the *Hpa*II restriction endonuclease junction between fragments 3 and 5 (refs 5 and 11). The sequence from this region can form either a stable hairpin loop or an interesting four-stranded conformation[11]. Moreover, it shows considerable homology with sequences from around the origins of replication of SV40 and BK virus. On the late side of the putative polyoma virus origin of replication, that is, within the 290 nucleotides that lie between positions 5,003 and 5,292 (Fig. 1), there are a number of AT-rich regions. These might facilitate the separation of the DNA strands through interaction with either an RNA molecule (see below) or a protein and allow the DNA to function at any given time essentially as a single-stranded species. Since the major mode of replication in polyoma virus is bidirectional and proceeds from a single site (or region) on each of the two strands, initial strand separation may be an important event in DNA replication.

Table 2 Calculated and measured molecular weights of polyoma virus proteins

Protein	Measured MW	(ref.)	Calculated MW*	Amino acids (no.)
VP1	45,000–48,000	(23, 24)	42,404	385
VP2	35,000	(24)	34,761	319
VP3	23,000	(24)	22,866	204
Large T antigen	88,000–100,000	(10, 56)	87,991	785
Middle T antigen	55,000	(57)	49,710 (48,566)†	432 (421)†
Small t antigen	22,000–23,000	(56)	22,785	195

*Based on DNA sequence.

†Depending on which splicing signals are used to produce the mRNA (see Fig. 2*b*).

The polyoma virus late mRNAs appear to consist of a noncoding 5′-'leader' sequence which, by some mechanism not yet understood, appears to be amplified, probably during transcription[31]. This sequence is attached to the main body of the mRNA in a process that involves (presumably) RNA splicing. The DNA sequence which corresponds to the late leader sequence appears to lie between nucleotides at positions 5,094 to 5,019. A part of this sequence, as deduced from a predicted ability to form base pairs, has a high degree of complementarity with the sequence which immediately precedes the initiation codon for VP3. That is, of the 46 nucleotides that lie between positions 5,092 and 5,046, 31 residues can form base pairs with the nucleotides that lie between positions 4,657 and 4,699. Moreover, part of the leader sequence can form a fairly strong secondary structure with the sequence which precedes the VP1 initiation codon[27]. Whether these long-range potential structures have any significance, and, if so, whether the significance lies at the DNA level, with DNA exercising control over transcription by these means, or at the RNA level, with precursor RNA controlling its own splicing (and thus translation), or with the mRNA controlling translation by its own secondary structure, is not known. It seems unlikely, however, that their potential presence within the genome is fortuitous.

If it is assumed that the sequence around the *Hpa*II-3/5 restriction endonuclease junction plays a part in viral DNA replication, and the sequence near the late coding region is of importance for the initiation of transcription, there are still about 150 nucleotides between positions 5,260 and 5,110 (Fig. 1) to which a function must be assigned. Within this region, the only assignment made (Table 1*b*) is to a potential promoter, that is, a sequence which resembles a so-called 'Hogness-box'. There is, however, a sequence which lies within this region which would allow the DNA, if transcribed, to produce an RNA which can adopt a cloverleaf configuration. This is illustrated in Fig. 3*b*. Such a structure was sought within the DNA because, as discussed above, it was apparent that if the UGA termination codon could be suppressed, a large amount of coding information would become available to polyoma virus. As some strains of *Escherichia coli* are known to contain a UGA suppressor, a tRNA^{Trp} (ref. 33), a search was made for a sequence in the virus which might specify a UGA suppressor. Since a viral gene specifying a tRNA might contain intervening noncoding sequences, such a search is not completely straightforward. One surprising result, however, was the considerable secondary structure found to be inherent in the DNA, and therefore to reflect structures which could exist in transcription products. The cloverleaf structure shown in Fig. 3*b* was one of the most stable structures observed and it could be formed from the nucleotides between positions 5,231 and 5,160. It does not, however, contain the appropriate anticodon for a UGA suppressor tRNA. The structure illustrated here as DNA has

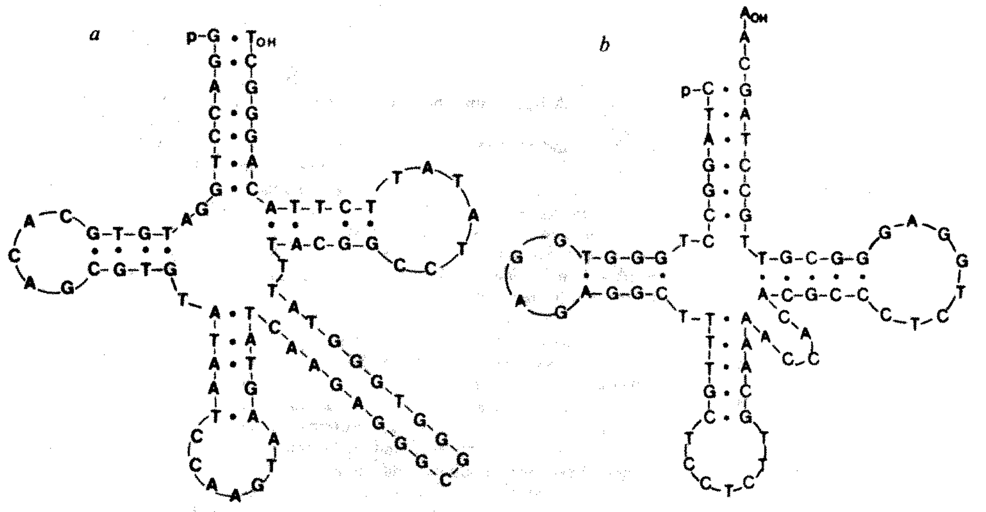


Fig. 3 *a*, Secondary structure of the DNA which lies in the early region of the genome between positions 715 and 799 (see Fig. 1). This region is of special interest because it appears to contain information which specifies some of the splicing signals in the early mRNAs[12,14]. *b*, An unusual clover-leaf structure allowed by the sequence of the DNA in a noncoding part of the polyoma virus late region, using nucleotides which lie in the late strand between positions 5,231 and 5,160. (It is doubtful, however, that the left hand loop, as such, would exist since it is stabilised by only two base pairs.) The anticodon loop has a similar, but not identical, sequence to postulated ribosomal binding sites[11,53] (see Table 1). An analogous kind of structure could be drawn for the complementary strand. This structure and the one above were found by a computer search using an unpublished computer program of R. Staden.

many, but not all of the features, of tRNA (for review see ref. 34). For example, it can be seen that the D-loop (left hand loop) could form hydrogen bonds with the TψC-loop (right hand loop). On the other hand, no tRNA has yet been described that has an anticodon loop composed entirely of pyrimidines (or on a corresponding structure from the other strand of the DNA, all purines). The comparison and contrast could be extended, but is of academic interest only unless it can be shown that polyoma virus codes for a tRNA. At present, it is not known whether the virus codes for any low molecular weight RNA molecules.

Apart from the question posed about viral tRNAs, nothing is known about the initial steps in viral DNA replication and transcription. It is conceivable that an RNA with a structure which corresponds to that shown in Fig. 3*b* might be involved in one or the other of these processes.

A comparison with SV40 and BK virus

It now appears that although much of polyoma virus resembles SV40, in some aspects the two viruses show profound differences. Since BK virus has been shown to be very similar to SV40 (refs. 35–37), much of what is said about the latter is presumably also true for the former.

Dealing first with similarities, the late coding regions in polyoma virus and SV40 have many features in common. Both viruses code for three late proteins which have similar sizes and appear to contain many homologous amino acids. Potential coding similarities between the two genomes themselves are illustrated in Fig. 2*a* and between their DNAs and predicted amino acids in Fig. 4*a* and *b* respectively. The latter also includes a comparison with BK virus. The greatest homology lies near the C-termini of the VP1 proteins. This conclusion, which is based on DNA sequences alone, is supported by the finding that the VP1 proteins of polyoma virus and SV40 are immunologically cross-reactive[38]; none of the other proteins has been found to be related in a similar way. In the region which contains the overlapping genes for parts of VP2/VP3 and VP1 (see Table 1*b*), 19 of the 32 base pairs (60%) in polyoma virus are homologous with nucleotides found in the similar position in SV40 (ref. 26). Although a region which codes simultaneously for parts of two proteins might be expected to be highly conserved in related viruses, the homology is, in fact, no greater than that which exists in similar lengths of sequence in many other parts of the genomes which code for one protein only, notably, for example, in regions near the N-termini of the large T antigens and the VP1 proteins. This may suggest that the sequence within the overlap is of little functional significance; on the other hand, it may emphasise the importance of retaining the sequence not only of the overlapping genes, but also in other regions in order to conserve diverse functions of the proteins. It has previously been argued, for example, that the quarter of large T antigen nearest its C-terminius may be important in viral DNA replication[12].

Over a large part (about 80%) of the early coding region, there is considerable homology between predicted amino acid sequences of two of the early proteins of polyoma virus and the two corresponding proteins (small t and large T antigens) of

Table 3 Codons used in polyoma virus genes

	U		C		A		G		
U	Phe	42	Ser	26	Tyr	25	Cys	27	U
		25		17		25		17	C
	Leu	22		25	Ochre	1	Opal	1	A
		22		4	Amber	2	Trp	29	G
C	Leu	40	Pro	38	His	27	Arg	3	U
		28		31		19		10	C
		51		42	Gln	37		9	A
		45		10		43		11	G
A	Ile	31	Thr	37	Asn	45	Ser	27	U
		9		35		23		33	C
		29	Thr	36	Lys	47	Arg	50	A
	Met	44		5		43		22	G
G	Val	31	Ala	47	Asp	50	Gly	23	U
		11		24		41		28	C
		28		37	Glu	55		47	A
		33		9		61		34	G

These data are taken from the coding sequences for VP1, VP2, middle T antigen and the part of large T antigen encoded in frame 2 (Fig. 2*a*). (All sequences present in VP3 are present in VP2 (ref. 24). Most of the sequence in small t antigen is also present in middle T antigen (refs 11, 15, 16), as is the frame 1 sequence of large T antigen.) These data are tentative since neither the exact splicing to create the mRNAs for middle T and large T antigens nor the sequence coding for the C-terminus of small t antigen are known. However, the data should at least be a very close approximation of the codons used. The bias against the use of CG is notable in codons specifying Ser, Pro, Thr and Ala but less notable in codons specifying Arg. Overall, this bias is much less than is observed in the case of SV40 (refs 7 and 8).

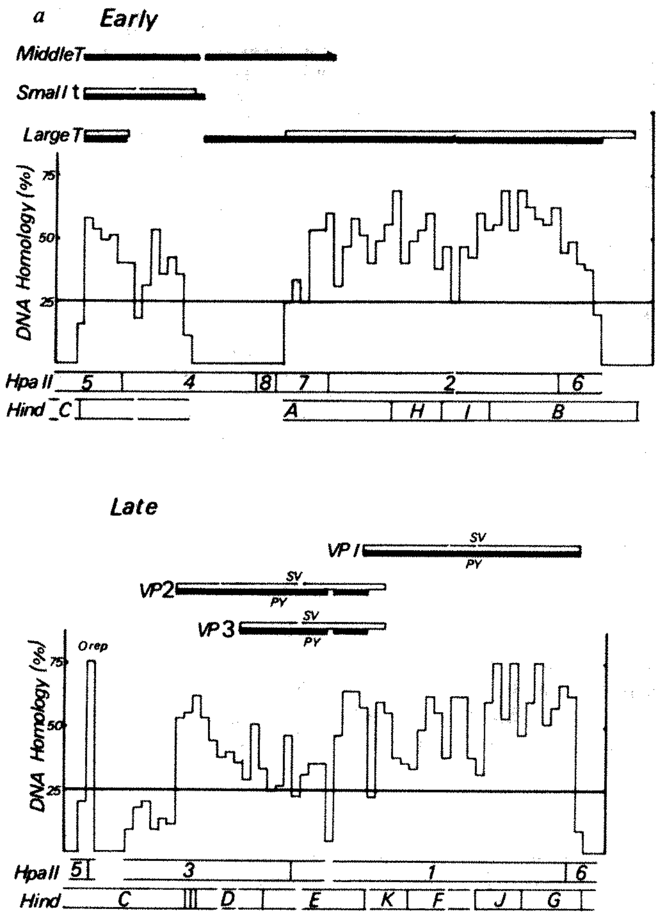
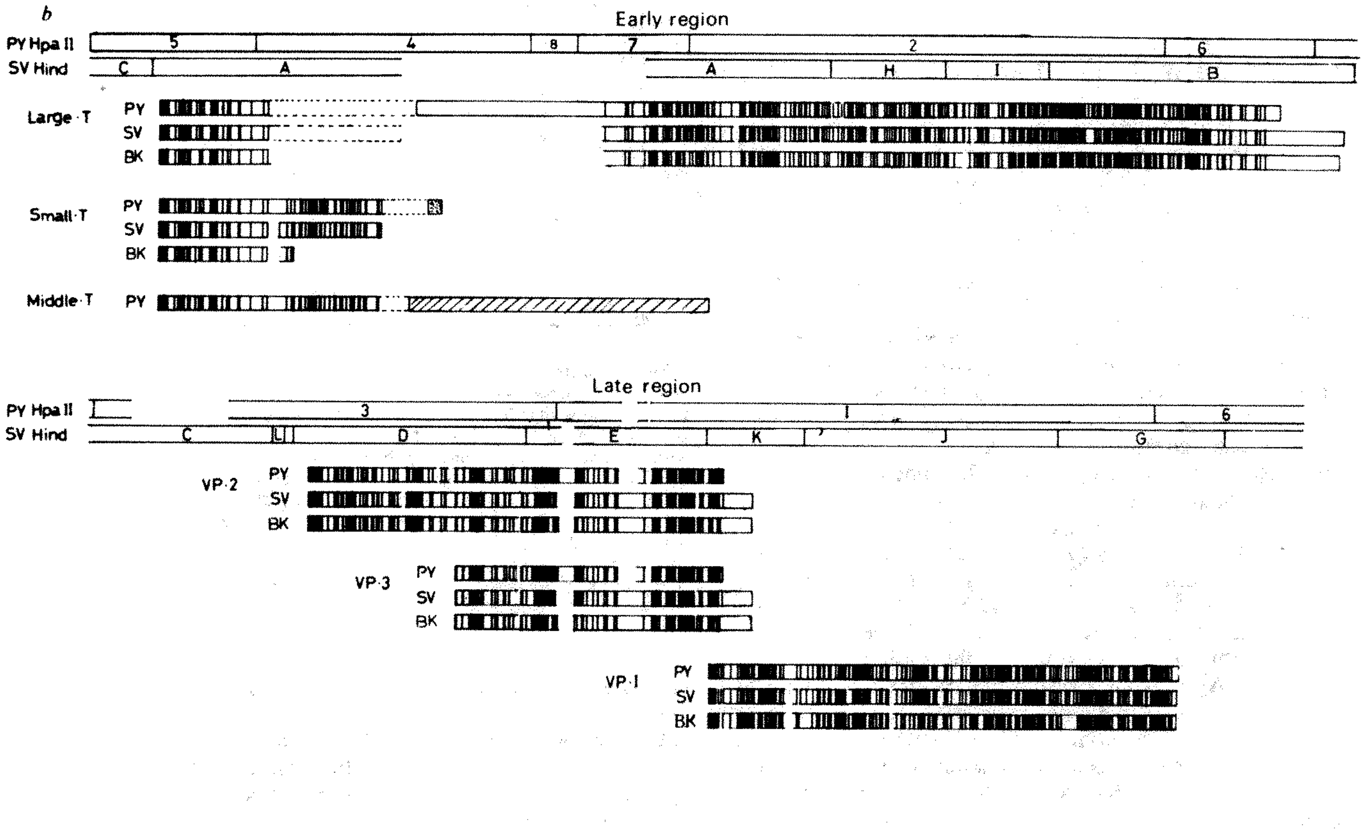


Fig. 4 *a*, A histogram which compares the homology in the genomes of polyoma virus and SV40, as derived from their DNA sequences (see Fig. 1 and refs 7 and 8). The polyoma virus and SV40 physical maps (Fig. 2*a*) are linearised about their respective origins of replication and divided into early and late regions. Gaps are left in the physical maps either where there is no corresponding sequence in the two genomes or where necessary in order to maximise homologies in adjoining regions. The location of the viral proteins relative to the genomes is shown. Percentage DNA homology, calculated for every 45 nucleotides, is plotted against sequence location in the genome. Homology becomes meaningful when it exceeds the 25% random expectation, and this is indicated by a solid line drawn across each figure. *b*, A comparison of the amino acid homology presumed to be present in individual proteins of polyoma virus and SV40. The polyoma virus and SV40 physical maps (see Fig. 2*a*) have been linearised about their respective origins of replication and divided into early and late regions, that is, regions coding for T antigens, and capsid proteins, respectively. From the DNA sequences (see Fig. 1 and refs 7 and 8), the amino acid compositions for each protein have been deduced and compared. A homologous amino acid occurring in the same protein among the two viruses is indicated by a black line (▌). Thus, black areas represent homologies and colourless areas non-homologies. Gaps are left whenever necessary in order to maximise homology. Dotted lines indicate regions of the DNA which are noncoding for any part of a particular protein under consideration. In a similar fashion, polyoma virus has been compared with the predicted protein compositions of BK virus (MM strain) as deduced from its DNA sequence[37]. The late region of SV40 has a number of duplicated sequences outside the coding region for which there is no counterpart in polyoma. This is indicated by a gap in the polyoma virus physical map. In the early region of polyoma virus DNA, there is a sequence which appears to have no counterpart in SV40. This is indicated in a similar manner by leaving a gap in the SV40 physical map. Neither SV40 nor BK virus appears to code for a protein which corresponds to the middle T antigen of polyoma virus, the body of which is encoded within the polyoma virus early sequence, indicated by cross-hatching (/ / /). The assessment of per cent homology between viruses is subject to the uncertainties inherent in the alignment of the genomes. When polyoma virus and SV40 are aligned in the manner shown here, leaving ocasional gaps but rearranging no sequences, genes coding for small t and large T antigens and the VP2 proteins had between 36 and 38% nucleotide homology, and the VP1 genes had about 53% homology (see *a*).



SV40, as shown in Fig. 4*b*. Similarity is also found in the overall organisation of the two early coding regions, as illustrated in Fig. 2*a* as well as in the regions immediately around their origins of replication[11]. However, nowhere in the two genomes does the homology over any length observable by electron microscopy exceed 60–70%. This may explain why earlier hybridisation studies failed to reveal homologies between the two viral DNAs and only in exceptional conditions was any heteroduplex formation observed[39]. In the latter case, the heteroduplex involved the DNA sequences which lie in a part of the polyoma virus and SV40 VP1 genes which code for the N-termini of the proteins and this is not even a region of exceptional sequence homology. Thus, earlier studies have probably not given a fair estimate of the similarities of the two viral genomes. This conclusion has been reached from studies on SV40 and its much closer relative, BK virus[36]. However, recent hybridisation studies, in less stringent conditions than previously used, do reveal limited regions of homology between polyoma virus and SV40 DNAs (ref. 40). Sequencing the genomes of similar viruses thus emphasises how little is actually known about the chemistry behind heteroduplex formation, or that involved in liquid hybridisation studies, and presents a more accurate account of relationships between species.

The similarities between polyoma virus, SV40 and BK virus are very striking and if these viruses evolved from a common ancestor, it becomes apparent which portions of the genomes have been conserved during evolution just as data discussed below may indicate changes in the genome which occurred during evolution. The differences among the viruses are also very noticeable (see Fig. 4) and may ultimately be found to be of considerable biological importance. These differences appear to lie in three parts of the genome: at the end of the early and late regions, and in a part of the early coding region. Considering first the ends of the early and late regions, SV40 has a large (about 2% of the genome) noncoding region between the portion of its genes which code for the C-termini of large T antigen and VP1 (refs. 7 and 8) (see Fig. 2*a*). No such large coding gap is found in the polyoma virus. In the latter, sequences which code for the C-termini of large T antigen and VP1 are separated by only seven base pairs. Further, in the noncoding regions around the viral origins of replication of the viruses, although there is considerable homology in the small portion of the sequences which may define the initiation of replication[11,41,42], there appears to be little sequence homology in the rest of these regions. Note that for polyoma virus, the region under consideration is somewhat less than 9% of the genome, whereas in SV40, it is about 13%. However, a search for homologies is somewhat hampered by the fact that much of the sequence in the noncoding part of SV40 consists of DNA repetitions[7,8]; a large part of the region can be deleted without affecting the viability of the virus[43]. It is not yet clear which sequence is redundant and which is essential in SV40. With polyoma virus, there appears to be much less, if any, analogous sequence redundancy. The noncoding regions around the origins of replication in the viruses may essentially be a control area of each virus. As such, it may be expected that the region might resemble a functionally related region in its host genome rather than another virus, even a related one, with a different host.

Finally, there also exists what currently appears to be the most important biological difference between the viruses, which is represented by the part of the early region in polyoma virus between about 86 and 99 units on the physical map. This region appears to code for the unique part of middle T antigen, as well as for part of large T antigen (refs 4 and 17). It seems that there is no comparable region in SV40 and without extensive sequence rearrangements, it is not clear how SV40 (or BK virus) could code for a protein comparable to the polyoma virus middle T antigen (see Figs 2 and 4). Studies with mutants indicate that this region in polyoma virus seems to be of critical importance in the virally induced transformation of cells and in tumour formation[4]. If polyoma virus has a gene which is responsible for the transformation of cells, and there is circumstantial though by no means conclusive evidence to suggest that it has, the middle T antigen is the best candidate for the product of this gene. If this proves to be the case and if SV40 does not code for a similar protein, then transformation may proceed by entirely different pathways, or involve different proteins, in the two viruses. An understanding of the differences between the two viral genomes may be essential in ultimately defining this property of the viruses. The known DNA sequences should now allow proper viral mutants to be constructed, by site-specific mutagenesis[44] or other procedures, which can establish the direct structural and functional relationships of these oncogenic viruses.

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Amino acid sequence changes in the haemagglutinin of A/Hong Kong (H3N2) influenza virus during the period 1968–77

W. G. Laver, G. M. Air, T. A. Dopheide* & C. W. Ward*

Department of Microbiology, John Curtin School of Medical Research, Australian National University,
PO Box 334, Canberra City, ACT, 2601, Australia

Haemagglutinin molecules from nine strains of A/Hong Kong/68 (H3N2) influenza virus, isolated between 1968 and 1977, were examined for changes in amino acid sequences. At least 18 changes, 9 of which were located precisely, occurred in the soluble tryptic peptides of the large haemagglutinin polypeptide (HA1) during this period. These peptides contained 262 residues (82% of HA1). In HA2, only two changes in 129 residues (58% of HA2) were detected. Sequential changes at a particular locus were not found; and as far as we can tell, once an amino acid changed, it did not change again in any subsequent variant examined.

INFLUENZA persists as an uncontrollable infection in man largely because of the high frequency with which antigenic changes occur in the surface proteins of the virus. Both influenza A and B viruses have two distinct surface antigens, the haemagglutinin (HA) and neuraminidase (NA). These may undergo a gradual change (antigenic drift) under increasing antibody prevalence in the population. Significant drift in the HA antigen is often associated with influenza epidemics of varying impact which occur without any regular time intervals. Occasionally, the HA and NA antigens of influenza A virus may abruptly undergo a complete change (antigenic shift) and bring about an influenza pandemic. Two recent influenza pandemics occurred, in 1957, when both the HA and the NA antigens changed ('Asian 'flu'), and in 1968, when only the HA antigen changed ('Hong Kong 'flu'). Influenza B viruses undergo antigenic drift, but are not known to undergo antigenic shift and have not been associated with pandemics.

Antigenic drift in type A influenza virus is believed to occur by the selection, in an immune population, of mutant virus particles with altered antigenic determinants on the HA and NA proteins. Peptide maps have shown that antigenic shift is associated with major differences in the amino acid sequence of the HA, and drift with minor differences[1].

Antigenic drift in the Hong Kong subtype

Since the appearance of the Hong Kong (H3N2) subtype of influenza type A virus in man in 1968, drift has occurred, resulting in the appearance of new variants, several of which are listed in Table 1. The original A/Hong Kong/68, and some of these variants, such as A/England/42/72, A/Port Chalmers/1/73, A/Victoria/1/75 and A/Texas/1/77, became widespread, causing epidemics in many countries, but most of the others were associated with only minor outbreaks and quickly disappeared[2]. Antigenic relationships between the viruses listed in Table 1 have been described previously[3,4].

In this study, we have examined the HAs of these nine Hong Kong variants for amino acid sequence changes, by matching the compositions of their soluble tryptic peptides with the known amino acid sequence of the homologous peptides from the Hong Kong variant, A/Memphis/102/72 (ref. 5).

The HA monomer is coded by a single, monocistronic segment of RNA[6,7] and is synthesised as a single polypeptide chain which is then normally cleaved by proteolytic enzymes to give two chains, HA1 and HA2, held together by disulphide bonds[8]. These two chains can be readily separated by column chromatography or density gradient centrifugation after denaturation and disruption of the disulphide bonds. HA1 is the N-terminal portion of the HA[9], has a molecular weight of 47,000 (ref. 10) and contains most of the carbohydrate[10,11]. HA2 is the C-terminal portion of the HA[9], has a molecular weight of approximately 30,000 (ref. 10), carries very little carbohydrate[10,11] and contains the hydrophobic tail by which the HA is attached to the viral membrane[9,12,13].

Sequence changes in HA1

Maps of the soluble tryptic peptides from HA1 of A/Hong Kong/68 and A/Memphis/102/72 viruses showed several differences[14]. Therefore, a detailed study of the changes occurring in nine different Hong Kong variants (Table 1) was carried out.

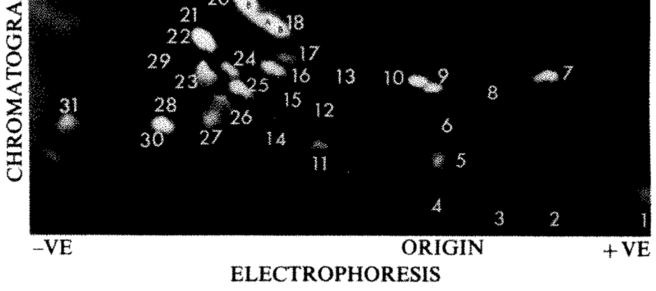

Fig. 1 Map of the soluble tryptic peptides from *S*-carboxymethylated HA1 of A/Memphis/1/71 influenza virus. Virus was grown in the allantoic sac of 11-d-old chick embryos and purified as described previously[22]. Haemagglutinin was isolated by electrophoresis of SDS-disrupted virus on cellulose acetate strips[19]. Heavy (HA1) and light (HA2) chains were separated by centrifugation on a guanidine hydrochloride–dithiothreitol density gradient[8]. The isolated chains were *S*-carboxymethylated as previously described[20], dialysed and precipitated with 3 volumes of ethanol. Digestion with trypsin (TPCK treated; Worthington), and mapping of the peptides soluble at *p*H 6.5 were as described elsewhere[14]. Peptides were located by staining with fluorescamine (10 μg per ml) in acetone containing 0.5% pyridine[21], eluted from the maps with 6 M HCl, hydrolysed in sealed, evacuated tubes for 22 h at 105 °C and analysed on a Beckman 119CL amino acid analyser. Tryptophan was qualitatively determined by staining duplicate maps with Ehrlich reagent. These methods were followed to prepare maps of HA1 and HA2 from the H3N2 viruses listed in Table 1. These viruses were grown as recombinants with the neuraminidase from A/Bel/42 (H0N1).

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* CSIRO, Division of Protein Chemistry, 343 Royal Parade, Parkville, Victoria 3052, Australia.

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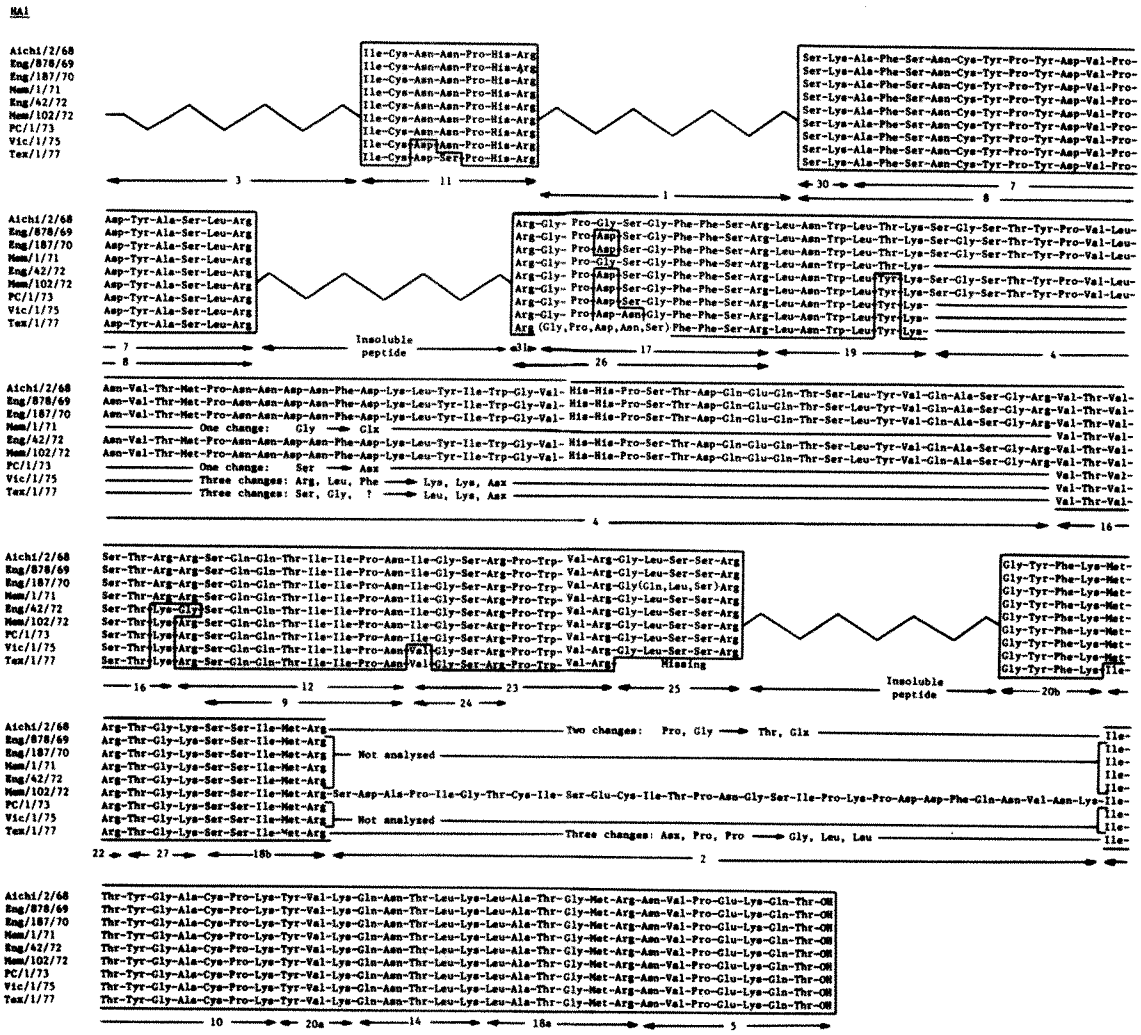
HA1
Aichi/2/68
Eng/878/69
Eng/187/70
Mem/1/71
Eng/42/72
Mem/102/72
PC/1/73
Vic/1/75
Tex/1/77
Insoluble peptide
Not analysed
Two changes: Pro, Gly → Thr, Glx
Three changes: Asx, Pro, Pro → Gly, Leu, Leu
One change: Gly → Glx
One change: Ser → Asx
Three changes: Arg, Leu, Phe → Lys, Lys, Asx
Three changes: Ser, Gly, ? → Leu, Lys, Asx
Missing

Fig. 2 Comparative partial amino acid sequences of the HA heavy chains (HA1) from nine different Hong Kong influenza A variants isolated between 1968 and 1977. The amino acid sequences were determined for the A/Memphis/102/72 strain (ref. 5 and C. W. W. and T. A. D., unpublished). Sequences for the other strains were deduced from the amino acid composition of their tryptic peptides. Boxed areas indicate regions of identical sequence and the residues which changed are indicated. It is not known which of the two Gly residues of Vic/75 peptide 17 changed to Ser in Texas/77, nor how one of the two Ser residues in Aichi/68 peptide 25 changed to Gln in Eng/187/70. Peptide numbers refer to the spots shown in Fig. 1. The four zig-zag lines denote regions of sequence associated either with the large peptides 1 and 3, which have not been fully sequenced and which therefore were not reliably analysed by single hydrolysis, or with two large insoluble tryptic peptides which we were unable to separate and analyse. The sequence of one of these (between peptides 25 and 20b) for the Mem/102/72 strain has been published[5] but no sequence data for this peptide from the other strains are available. We have previously found that variants of A/Memphis/1/71 virus selected with monoclonal antibodies showed single amino acid sequence changes[15]. These were in peptide 11, where the Asn at position 4 changed to Lys; peptide 16, where the Ser changed to Tyr; peptide 17 (26) where the Pro changed to Ser, Leu, Thr or His, and peptide 25, which was missing in one monoclonal variant, although the change responsible was not determined.

Figure 1 shows a map of the soluble tryptic peptides from *S*-carboxymethylated HA1 of A/Memphis/1/71. The assumed sequences and locations of these A/Memphis/1/71 peptides, based on their amino acid composition[15] and the available sequence data for the closely related A/Memphis/102/72 strain[5], are shown in Fig. 2 with the corresponding data for the soluble HA1 tryptic peptides from the other eight Hong Kong influenza A variants examined.

Figure 2 shows that most of the primary structure of Hong Kong virus HA remained unchanged during the period 1968–77. In the soluble tryptic peptides, which contained 262 amino acids (82% of HA1), 18 changes in sequence could be detected. Of these, nine were located precisely, but in the four large peptides (1, 2, 3 and 4), where changes in composition were seen, we cannot know the exact number of sequence changes without more detailed analysis of sub-fragments. There may be more changes in sequence than detected by composition analysis. The regions of sequence not covered in this report are associated with two large insoluble tryptic peptides that will require additional enzymatic digestion before sequence analysis.

In most cases, once an amino acid residue had changed, it did not change again in any of the other variants subsequently isolated. Exceptions to this were seen in four cases where apparent reversion to the original amino acid was seen. Thus, in peptide 12, the arginine in position 1 in A/Aichi/2/68, A/England/878/69, A/England/187/70 and A/Memphis/1/71 was replaced by glycine in A/England/42/72 but is arginine in the subsequent strains. A likely explanation is that this isolate of A/England/42/72 was not the virus that gave rise to the 1972–77 variants. As reported previously[1], peptide 25 from HA1 of A/Queensland/7/70 virus had the leucine residue replaced by glutamine. Although this change was not found in

Table 1 Hong Kong (H3N2) variants examined

A/Aichi/2/68	A/Memphis/102/72
A/England/878/69	A/Port Chalmers/1/73
A/England/187/70	A/Victoria/1/75
A/Memphis/1/71	A/Texas/1/77
A/England/42/72	

All these viruses were obtained from WHO reference laboratories, with the exception of the Memphis strains, which were local isolates. The viruses were grown as recombinants with the neuraminidase from A/Bel/42 (H0N1). The recombinants were cloned twice at limit dilution before analysis.

any of the Hong Kong strains examined in this report, the Eng/187/70 strain had a Ser → Gln change in the same peptide (see Fig. 2). The most likely explanation is that the A/Queensland/7/70 and Eng/187/70 strains did not persist in nature and did not give rise to subsequent strains. The third example is in peptide 4, where in Mem/1/71 there is a Gly → Gln change not seen subsequently. The fourth example of apparent reversion back to the original residue can be seen in peptides 17/26 where the glycine at position 4 is replaced by aspartic acid in the 1969 and 1970 England variants, but not in the 1971 American strain, A/Memphis/1/71. All subsequent variants (1972–77) have aspartic acid in this position. Although the nine variants studied here represent Hong Kong variants isolated in chronological order, they do not necessarily represent an exact geneological series. Variants showing this Gly → Asp change in peptides 17/26 apparently arose first in England, then spread to, or arose independently in, the US.

Almost all the changes we have observed in compositions of small peptides can be accounted for by a single nucleotide change. One exception is in peptide 25 of Eng/187/70 (and no other strain we have examined), where there is a Ser → Gln change. It would be interesting to sequence this peptide, because the sequence could show two changes, for example,

A A
CUG UCG

Gly - Leu - Ser - Ser - Arg

↓ ↓

Gly - Gln - Leu - Ser - Arg

A A
CAG UUG

The Leu → Gln change has been found in Qld/7/70, although peptide 25 in subsequent strains is the same as in 1968 and 1969 strains until Texas/1/77, where the peptide disappears (indicating some other change).

The other 2-base change appears in peptide 19 of Eng/42/72 and Mem/102/72, and persists through to Texas/1/77, being Thr → Tyr

$$\mathrm{ACX} \rightarrow \overset{\mathrm{U}}{\mathrm{UAC}}$$

In the large peptides 4 and 2, several of the observed changes in composition would require at least two nucleotide changes in the RNA.

Sequence changes in HA2

Figure 3 shows the map of the soluble tryptic peptides obtained from *S*-carboxymethylated HA2 of A/Memphis/1/71 virus. Using the sequence data available for A/Memphis/102/72 (ref. 5) it was possible to deduce the sequences of most of these A/Memphis/1/71 peptides from their amino acid compositions[15]. The location of each of these peptides in the partial sequence of HA2 is shown in Fig. 4. These peptides account for 129 residues (58% of the total HA2 sequence), the rest being insoluble and not examined.

Although we have not been able to characterise the exact changes, the data presented in Fig. 4 show that in the 20 soluble HA2 peptides examined, only two differences could be detected between the first and last Hong Kong strains. This suggested that extensive variation would not be found in the intermediate strains, and therefore these were not examined.

Relationship between sequence changes and antigenicity

The analysis of amino acid sequence changes in natural influenza variants may not necessarily indicate which portions of the sequences make up the antigenic determinants. Some of the changes observed may be unrelated to the antigenic differences between the various HAs. Considering the large antigenic difference between Hong Kong/68 and Texas/77 viruses[3], and the large number of antigenic sites on the HA molecule[16], the number of sequence changes found associated with antigenic drift was surprisingly small. In most cases, only one or two amino acid changes were seen between neighbouring drift strains. The heavy chain (HA1) from the last member of the series, A/Texas/1/77, differed from that of the first, A/Aichi/2/68, by about 18 out of 262 residues, whereas the light chain (HA2) showed only two amino acid substitutions in the soluble tryptic peptides during antigenic drift in this period.

We do not know which of the changes in sequence found to occur between 1968 and 1977 were responsible for the changes in antigenicity which occurred in the Hong Kong virus during this period. In a previous study[15], influenza variants of A/Memphis/1/71 were selected with monoclonal hybridoma antibodies and examined for changes in amino acid sequence and antigenicity. These variants showed dramatic changes in antigenicity, having completely lost the capacity to bind the monoclonal antibody used for their selection. These changes were found to be associated with a single amino acid substitution in HA1, which in every case required only a single base change in the RNA. No changes were seen in HA2. The HA1 tryptic peptides in which alterations occurred in the variants were the same as those in which changes occurred in the field strains, but in every instance the amino acid changes in the monoclonal variants were different from those found in the field strains (Fig. 2).

The recombinant viruses we used as the source of HA were cloned twice at limit dilution before analysis; this may have led to the separation of variant viruses with sequence changes in the HA. Furthermore, HA molecules of viruses isolated late in the Hong Kong era, antigenically related to the 'Victoria' and 'Texas' strains, may well have a number of sequence changes from the Victoria and Texas strains we examined. Asian (H2N2) viruses isolated in different parts of the world in 1968 at the end of the H2N2 period showed considerable differences in their HA1 and HA2 tryptic peptide maps[17].

Electron microscopy of antibody molecules attached to the HA has shown that some (and possibly all) of the antigenic determinants are situated in a region just below the tip of the HA spike[18]. In the present work we have found changes along the whole length of the HA polypeptides, which may reflect the way in which the polypeptide chains are folded within the HA spike.

On the other hand, we do not know which of the sequence changes are responsible for the antigenic differences between the various viruses and which are not. Neither do we know if the sequence changes responsible for the antigenic changes occur in

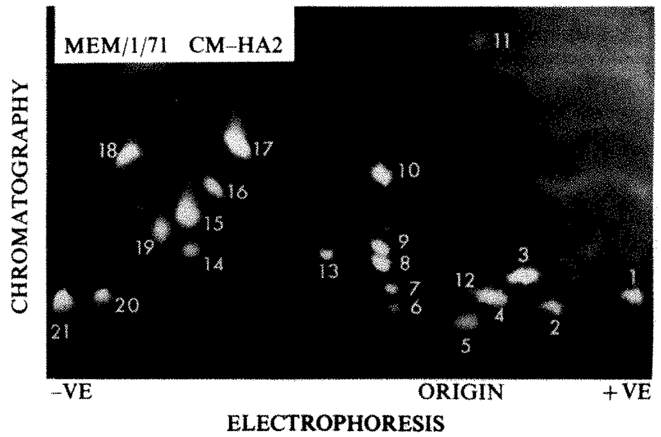

Fig. 3 Map of the soluble tryptic peptides from *S*-carboxymethylated HA2 of A/Memphis/1/71 influenza virus. The methods for virus growth, HA2 purification, enzyme digestion and peptide mapping are described in Fig. 1 legend.

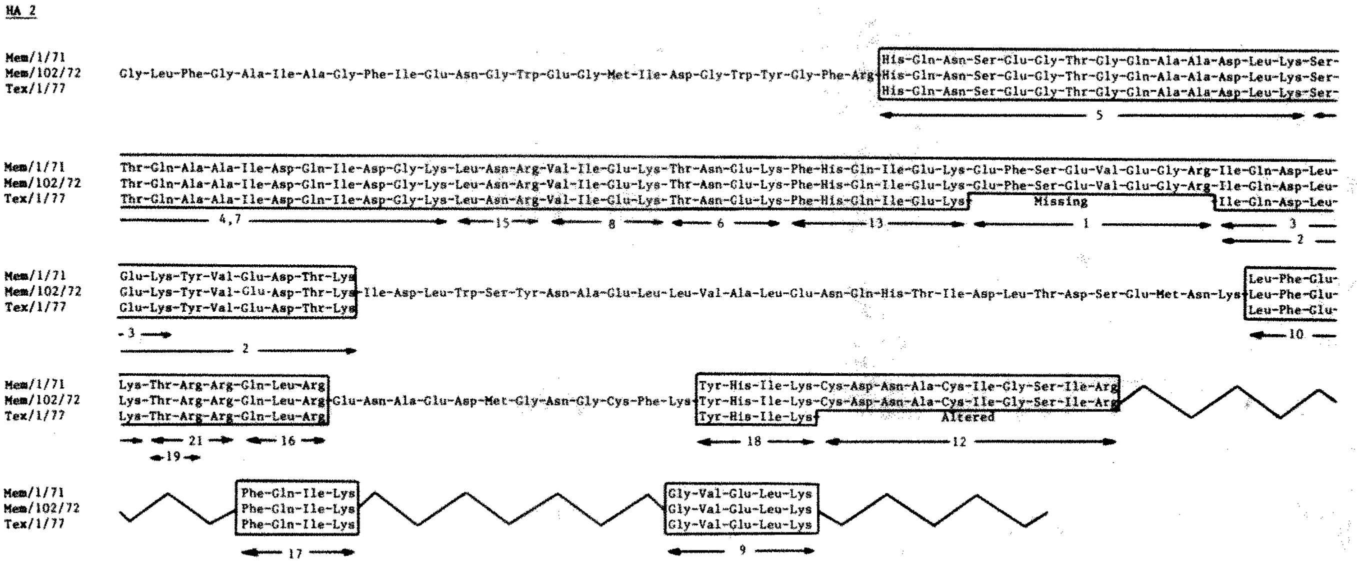
HA 2
Mem/1/71
Mem/102/72
Tex/1/77
Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-Phe-Ile-Glu-Asn-Gly-Trp-Glu-Gly-Met-Ile-Asp-Gly-Trp-Tyr-Gly-Phe-Arg
His-Gln-Asn-Ser-Glu-Gly-Thr-Gly-Gln-Ala-Ala-Asp-Leu-Lys-Ser-
5
Thr-Gln-Ala-Ala-Ile-Asp-Gln-Ile-Asp-Gly-Lys-Leu-Asn-Arg-Val-Ile-Glu-Lys-Thr-Asn-Glu-Lys-Phe-His-Gln-Ile-Glu-Lys-Glu-Phe-Ser-Glu-Val-Glu-Gly-Arg-Ile-Gln-Asp-Leu-
Thr-Gln-Ala-Ala-Ile-Asp-Gln-Ile-Asp-Gly-Lys-Leu-Asn-Arg-Val-Ile-Glu-Lys-Thr-Asn-Glu-Lys-Phe-His-Gln-Ile-Glu-Lys
Missing
Ile-Gln-Asp-Leu-
4,7
15
8
6
13
1
3
2
Glu-Lys-Tyr-Val-Glu-Asp-Thr-Lys
Ile-Asp-Leu-Trp-Ser-Tyr-Asn-Ala-Glu-Leu-Leu-Val-Ala-Leu-Glu-Asn-Gln-His-Thr-Ile-Asp-Leu-Thr-Asp-Ser-Glu-Met-Asn-Lys
Leu-Phe-Glu-
3
2
10
Lys-Thr-Arg-Arg-Gln-Leu-Arg
Glu-Asn-Ala-Glu-Asp-Met-Gly-Asn-Gly-Cys-Phe-Lys
Tyr-His-Ile-Lys-Cys-Asp-Asn-Ala-Cys-Ile-Gly-Ser-Ile-Arg
Tyr-His-Ile-Lys
Altered
21
16
19
18
12
Phe-Gln-Ile-Lys
Gly-Val-Glu-Leu-Lys
17
9

Fig. 4 Comparative amino acid sequences of A/Memphis/1/71, A/Memphis/102/72 and A/Texas/1/77 HA light chains (HA2). The amino acid sequences have been determined for the A/Memphis/102/72 strain[5]. Sequences for the other two strains were deduced from their amino acid compositions. The peptide numbers refer to the corresponding spots shown in Fig. 3. The regions of residues 1–25 and 89–117 and much of the C-terminal sequence involve large insoluble tryptic peptides not analysed in this study. In peptide 18 the sequence has been revised and corrected from that given previously[5].

those amino acids which constitute the antigenic determinants, or whether the sequence changes occur elsewhere in the molecule and cause conformational changes which alter the antigenic determinants.

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LETTERS

IR sources in the Southern Coalsack

M. J. Smyth
University of Edinburgh, Department of Astronomy, Edinburgh

M. E. Sim
Royal Observatory, Edinburgh, UK

A preliminary survey of four dark globules in the Southern Coalsack, an area of conspicuous obscuration in the southern sky, has revealed seven IR sources, which are most probably heavily obscured background stars. We report here that the wavelength dependence of extinction is not significantly different from that in the general interstellar medium. Spectroscopy of these stars, and of still more heavily obscured stars that should be revealed by a deeper survey, would permit detailed analysis of 'proto-protostellar' material. Although these are probably background stars, the numbers found in our small sample at least suggest association with the globules themselves; there is growing evidence for star formation within or associated with dense dark globules.

Bok[1] has reviewed the important role of dark globules in our understanding of star birth in the Galaxy, and has suggested the use of IR photometry to probe these heavily absorbing 'proto-protostars'. The Southern Coalsack, the finest dark nebula in the southern Milky Way, contains many globules, which appear as roundish, sharply bounded dark nebulae a few arc-min in diameter. Bok, Sim and Hawarden[2] have superimposed blue–green and far-red photographs of Globule 2 of the survey of Tapia[3], the far-red images revealing peripheral reddened background stars invisible on the blue–green photographs.

We have used the 0.75 m telescope of the South African Astronomical Observatory to make a quick survey of four Coalsack globules, numbers 1, 2, 3 and 9 in Fig. 1 of ref. 2, at a wavelength of 2.2 μm (*K* magnitudes). The areas scanned were squares of 7 × 7 arc min in each case. For illustrations we again used two photographs taken with the SRC 1.2 m Schmidt telescope at Siding Spring, Australia. Their star images were almost superimposed in a modified Zeiss blink comparator, and Fig. 1 shows photographs of the four globules taken from the TV monitor. Black images correspond to stars in the far-red photograph ($\lambda \sim 0.8$ μm), and displaced white images to those in the blue–green photograph ($\lambda \sim 0.5$ μm); again, far-red images unaccompanied by blue–green images can be seen within the globule boundaries, representing heavily reddened background

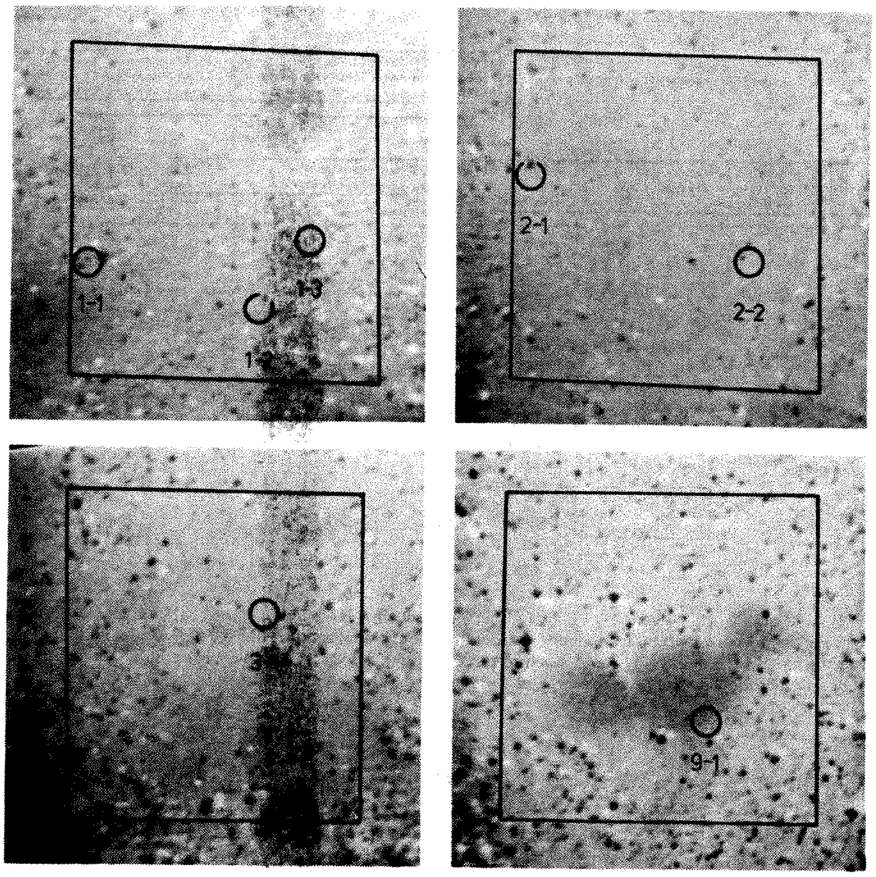

Fig. 1 TV monitor displays of the globules observed. Squares (7 × 7 arc min) show the areas scanned, and circles the locations of IR sources found. North is at the top, east to the left. Images from the far-red photograph are shown in black, those from the blue-green photograph in white.

stars. Boundaries of the square areas scanned are indicated, and circles enclose the locations of the brighter IR sources found, whose positions are listed in Table 1. In no case was any star visible in the photometer fields centred on the sources. The 3σ detection limit of the scans was $K \sim 8.5$. Several sources were detected, and results of subsequent J (1.25 μm), H (1.6 μm), K (2.2 μm), L (3.4 μm) photometry of the brighter ones are given in Table 2. Errors are of order 0.03 mag for J, H, K and 0.1 mag for L.

On the assumption that these are background stars whose light has been reddened by passage through the globules, we compare in Fig. 2 their (J–H, H–K) colours with those of an unreddened $M0$ star[4]. They lie on a locus close to that predicted by the normal interstellar extinction law (Table 12 in ref. 5). It is statistically most likely that random background stars are cool giants of spectral type K or M, but the assumption of early spectral type would force the extinction line through the origin and imply an abnormally steep slope. Elias[6] also finds a normal extinction law in studies of extended dark cloud complexes.

To use the normal law to deredden the observed colours we require spectral types, giving intrinsic colours. In the absence of IR spectrophotometry we have estimated the probable range of total absorption on the basis of a range of spectral types from $G5$ to $M5$; the resulting ranges of total visual absorption A_V and intrinsic apparent magnitude V_0 are listed in Table 2. With the exception of source 1–1 near the edge of a scanned area, the total absorptions almost certainly exceed 10 mag. Note that these heavily obscured sources were the brightest objects located in the scans at 2.2 μm.

Table 1 Location of brighter IR sources

Source	α (1975)	δ (1975)
1–1	12 h 32 min 28 s	−63°34′.4
1–2	12 h 31 min 52 s	−63°35′.3
1–3	12 h 31 min 42 s	−63°33′.8
2–1	12 h 30 min 33 s	−63°35′.7
2–2	12 h 29 min 47 s	−63°37′.4
3–1	12 h 28 min 39 s	−63°39′.1
9–1	12 h 34 min 39 s	−63°04′.2

These quite large absorptions are found for stars that lie 1 or 2 arc min from the centres of the globules, where presumably the absorption is considerably higher. Bok[1] has conjectured that core absorptions at 1 μm do not exceed 7 mag, implying $A_V \leqslant 19$; our observations of peripheral IR sources suggest that higher central absorptions may occur. From star-counts, Bok *et al.*[2] estimated $A_V \sim 5$ in an outer annulus of Globule 2.

The ranges of deduced intrinsic magnitudes in Table 2, in conjunction with absolute magnitudes of giant stars from $G5$ to $M5$, imply distance moduli between 5.5 and 12, that is distances between 125 and 2,500 pc. The stars could thus lie within the

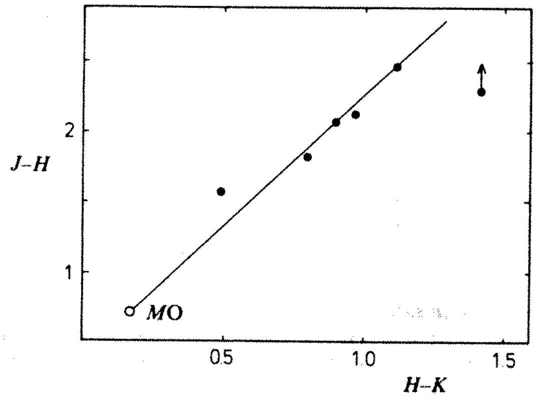

Fig. 2 IR colour indices $J-H$, $H-K$ for six of the heavily reddened sources, compared with those of a normal $M0$ star. The slope of the reddening line is 1.8, close to the normal interstellar value.

Table 2 Deduced intrinsic magnitudes

Source	J	H	K	L	A_V	V_0
1–1	8.23	6.65	6.16	5.94	6.5–9.5	7–12
1–2	9.72	7.58	6.61	6.03	13–16	7–11
1–3	10.03	8.20	7.40		10–13	7–11
2–1			8.60			
2–2	10.09	7.61	6.49	5.88	16–19	7–11
3–1	9.15	7.07	6.17	5.56	12–15	7–11
9–1		9.53	8.11			

globules (the estimated distance of the Coalsack is 175 pc) but more probably lie beyond them. On the other hand, the cumulative source–count model of Elias[6] gives a probability of ~0.3 for finding a star intrinsically brighter than 7.5 (or $K < 8.5$ with $A_K = 1$) within one of the scanned areas, whereas in fact seven sources were found in four areas. It is thus tempting to suggest that the heavily reddened stars are physically associated with the globules. There is indeed increasing evidence for star formation associated with globules: Bok[7] supports this view and gives an example of Herbig–Haro objects located within a dense globule. Our preliminary survey, in revealing the presence of intrinsically bright stars, and unexpectedly high absorption densities, again points in the direction of recent star formation in some globules.

We have surveyed only four of our Galaxy's population of dark globules, and cannot draw strong conclusions from this small sample. Evidently IR photometry should be used to probe a significant fraction of the observable globules. High-resolution IR spectrometry should be attempted for the brighter sources located, to provide absorption spectra of proto-protostellar material.

For locating heavily reddened stars, array detectors working at shorter wavelengths may ultimately prove more powerful than single detectors scanning at, for example, 2.2 μm. At 1 μm the very large number of picture elements and high detection efficiency of a silicon CCD array will outweigh the lower globule absorption at 2.2 μm, except perhaps in the densest globule regions. R. Albrecht, B. J. Bok and G. Gozza (personal communication) have experimentally photographed Coalsack Globule 2 with such a device, and have indeed recorded stars close to the centre. But we have demonstrated that, until such devices become more widely available, scanning with an efficient InSb detector produces valuable results with a reasonable expenditure of telescope time.

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Coronal Faraday rotation during solar occultation of PSR0525+21

M. K. Bird, E. Schrüfer & H. Volland

Astronomical Institutes, University of Bonn, Auf dem Hügel 71, 5300 Bonn, FRG

W. Sieber

Max Planck Institute for Radio Astronomy, Auf dem Hügel 69, 5300 Bonn, FRG

The apparent position of the pulsar PSR0525+21 is within 1.3° (4.72 $R_\odot$) of the south solar pole on or about 13 June of each year. During these times of superior conjunction, the linearly polarised component of the pulsar radiation is subjected to an additional Faraday rotation due to the high electron density and strong magnetic field of the solar corona. We report here measurements of this coronal Faraday rotation in 1978 and 1979 at the 100-m radio telescope of the Max-Planck-Institut für Radioastronomie in Effelsberg which compared pulsar polarisation profiles at $\lambda = 17.4$ cm ($f = 1{,}720$ MHz) for times before, during and after occultation by the Sun. A significant residual rotation measure, which exceeded the interstellar value, was observed both years at times close to that of minimum solar approach.

Pulsars were recognised early as potential sources of probe signals for coronal investigations[1,2]. Group delay time measurements, from which one may determine coronal dispersion measure (DM) by recording arrival times of pulses in well separated frequency bands, were first performed using the Crab Pulsar in 1969 at NRAO[3] and Arecibo[4]. The additional delay in arrival time is proportional to the columnar electron content in the corona along the ray path pulsar/Earth, and is a very small fraction ($\simeq 10^{-4}$) of the total delay due to interstellar dispersion. The Arecibo experiment was repeated in 1970, 1971 and 1973 to determine changes in the corona at later phases of the solar cycle[5,6]. Higher electron densities were measured at polar heliographic latitudes during solar maximum than during solar minimum.

In addition to measurements of coronal DM, a linearly polarised source could be used to determine the coronal rotation measure (RM) simply by tracking the source polarisation angle during occultation. The additional Faraday rotation of the plane of polarisation is proportional to the integrated product of electron density times the component of magnetic field along the line-of-sight in the corona. Values comparable with interstellar rotation measures can be achieved at solar elongations ≤ 2°. An experiment of this type was first attempted by Golnev *et al.*[7] in 1962 using the Crab Nebula. Successive experiments on the same source were carried out by Sofue *et al.*[8] and Berlin *et al.*[9]. Passive Faraday rotation experiments were also performed at low heliographic latitudes using the linearly polarised carrier signals of Pioneer 6 (ref. 10) and the two Helios spacecraft[11].

The first coronal Faraday rotation experiment using a pulsar as the source of the probe signal is described here. One decisive advantage of pulsars over continuously emitting sources is the built-in compensation for background noise (mainly from solar sidelobe interference), which can sometimes be several orders of magnitude more intense than the pulsar. The background is eliminated with a measurement during the 'off' phase of the pulsar period without having to drive the antenna off source. Preliminary measurements were taken in January 1978 when the pulsar PSR2045−16 came to within 5.6 $R_\odot$ of the Sun[12]. The results of this first run were indicative, but not entirely convincing, that a coronal contribution was present.

Reliable measurements of coronal Faraday rotation were obtained, however, at the June occultations of PSR0525+21 in 1978–79. The occultation geometry for the two years is shown in Fig. 1. The apparent position of PSR0525+21 moves by the Sun from east to west at 1° per day in a plane parallel to the ecliptic. The UT start of days 11–17 June is marked by the series of large dots and the observation intervals are indicated by the heavy bars along the path. The paths of the intrinsically weaker pulsar PSR0540+23, which was used primarily as an ionospheric sounder in this experiment (see below), are also shown.

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A stable integrated pulse profile of PSR0525+21 and two non-occulted 'control' pulsars (PSR0540+23 and PSR0823+26) was established using measurements for times well separated from the Sun. This profile, consisting of total intensity, linear intensity and polarisation angle at 256 equally spaced points across the pulse window, was termed the 'master' profile. During solar occultation the pulses were integrated just long enough to obtain what was felt to be a stable 'test' profile, which ideally differed from the master profile only in the absolute polarisation angle. This difference, after compensation for possible ionospheric effects, was attributed to the interposed solar corona. Only points within the test and master pulse windows with a high degree of linear polarisation were used in the determination of the mean weighted polarisation angle difference for a given test profile. The accuracy in determining this differential Faraday rotation is a function of integration time, solar offset (background noise) and intrinsic source intensity during the recording of each test profile. A typical error using this technique (one standard deviation of the weighted mean coronal rotation measure) was of the order of ±1 rad m^{-2}.

When possible, the correction for the ionosphere was made using observations of PSR0540+23, whose ray path penetrates virtually the same ionospheric regions as that of PSR0525+21. It was originally thought that PSR0540+23 was not sufficiently intense for coronal sounding measurements. However, aside from the scintillation fading that occasionally degraded the signals of all pulsars, PSR0540+23 was determined *a posteriori* to be only slightly worse adapted for this experiment than PSR0525+21. The minimum offset distance for PSR0540+23 is the smallest of all known pulsars[13], and is the only one available for coronal sounding at low heliographic latitudes.

The coronal rotation measure of the signal from PSR0525+21 on 12–15 June 1978–79 (ionospheric correction included) is presented in Fig. 2. One degree of Faraday rotation yields a rotation measure of 0.58 rad m^{-2} at the observing frequency of 1,720 MHz. The apparent distance of the pulsar from Sun centre (solar offset) is given for both years at the top of the figure.

The data for the two years are remarkably similar, particularly when one considers the adjustment for the two solar offset scales. A large negative Faraday rotation (mean coronal field directed away from observer), essentially symmetric about the point of closest approach, was recorded on both occasions. This coronal contribution first becomes large at solar offsets $<5\ R_\odot$, which is approximately the solar distance at which the solar wind undergoes a transition from a rapidly divergent flow (number density n goes as r^{-6} in semi-empirical models) to one which is purely radial ($n \sim r^{-2}$). This may help to explain the undramatic observations of PSR2045–16 in January 1978 (ref. 12), which did not approach the Sun closely enough. The magnitude of the coronal effect in Fig. 2 was comparable with that recorded in the ecliptic at the same solar offset even though the polar regions of the Sun are dominated by considerably less dense coronal holes[10,11].

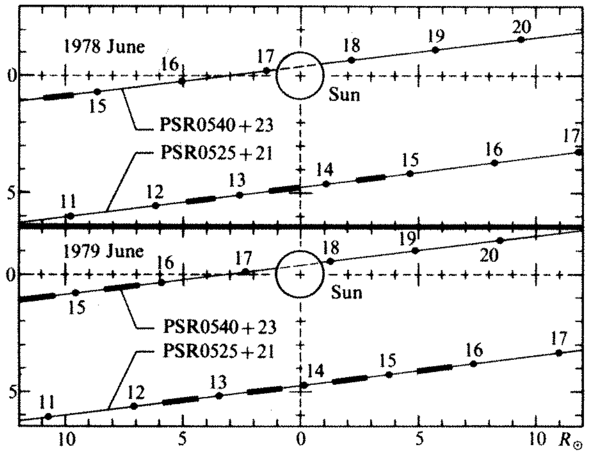

Fig. 1 Apparent paths of the pulsars PSR0525+21 and PSR0540+23 during their solar occultations in June 1978–79. The ray path pulsar/Earth moves parallel to the ecliptic from east to the west solar limb in this solar equatorial projection. The dots mark the start of a UT day, the associated number giving the June date for each year. The heavy bars along the path denote the observation intervals at the Effelsberg telescope.

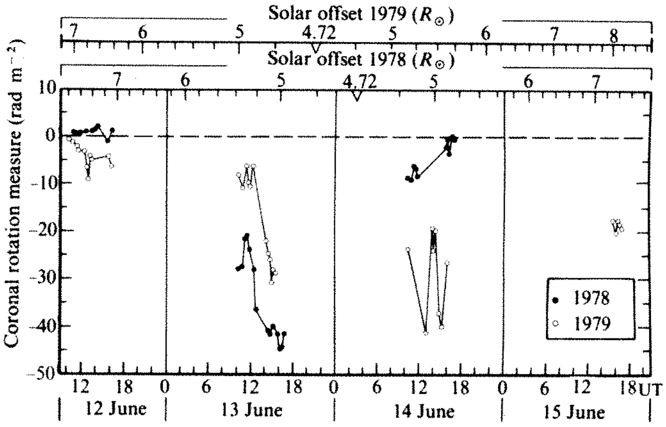

Fig. 2 Coronal rotation measure of the signal from PSR0525+21 during its solar occultations in 1978–79. The solid (open) points display the results obtained in June 1978 (1979). The minimum apparent solar offset of PSR0525+21 (4.72 $R_\odot$) is attained at a slightly different UT each year as shown in the two scales at the top.

The solar rotation measure is seen to be a continuous even function about the point of closest approach, so it may be assumed that the main contribution stems from a monopolar magnetic region situated over the south pole. This contrasts with previous spacecraft occultations in the ecliptic[10,11], where a field reversal about the mid-point of the occultation was observed (odd functions). As the south solar pole has a towards polarity in this phase of the present solar cycle, regions on the near side of the Sun (viewed from Earth) will cause the negative Faraday rotation that is observed. It is concluded therefore that either the polar hole region is greater in extent or contains higher electron density times magnetic field on the nearer Earth leg of the coronal ray path.

These observations have demonstrated that pulsars are the best natural sources available for the coronal Faraday rotation experiment. The highly polarised pulsed emission could be used for simultaneous recording of solar DM and RM, although no single ground facility is ideally instrumented for such observations. It is planned to coordinate a joint observational programme at Effelsberg and Arecibo for the 1980 tandem solar occultation of PSR0525+21 and PSR0540+23, which will occur during the Solar Maximum Year (SMY) and would thus profit from independent support not only from other ground-based observatories, but also from the Solar Maximum Mission (SMM), which will be in full operation by that time.

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Photo-sputtering of ice and hydrogen around Saturn's rings

R. W. Carlson

Earth and Space Sciences Division, Jet Propulsion Laboratory,
California Institute of Technology, Pasadena, California 91109

Rocketborne UV spectroscopic measurements have shown that a surprisingly dense cloud of atomic hydrogen surrounds Saturn[1,2] which has been interpreted as being associated with the Saturn ring system. Verification of an UV emission cloud related to the rings is given by Pioneer 11 flyby measurements[3]. Several ring sources of atomic hydrogen have been proposed[4–7], but abundance and lifetime estimates given below require a source which is much more potent than those previously considered. An alternate mechanism which satisfies this source requirement is proposed here, and involves photodissociation of surface water molecules on the icy ring material.

The rocket measurements of Weiser *et al.*[1,2] show hydrogen Lyα emissions outside the disk of Saturn with a mean brightness of 200R ± 50%. If this emission is produced by resonance scattering of the incident solar Lyα line with an efficiency[8] of 2.3×10^{-5} s^{-1} atom^{-1}, then the total number of atoms within their viewing aperture is $N\approx8\times10^{33}$ atoms (assuming optically thin conditions). The mean density is $n\approx400$ cm^{-3}. One can relate this abundance to a production rate through the hydrogen atom lifetime as discussed below.

Loss of H atoms can be caused by many processes, including photoionisation and charge exchange. However, the major loss of H atoms produced in, or near, the rings is through collisions and absorption by ring particles. Laboratory measurements[9] of the reflection of H atoms by low temperature ice show reflection coefficients of 78%, that is, 22% of the collisions will result in loss of a hydrogen atom. The low density of the gaseous ring 'atmosphere' implies that molecular collisions are infrequent and that atoms follow keplerian orbits about Saturn. The trajectories of atoms created near the rings will pass through the rings generally once per orbit, twice if the orbital eccentricity is small. As the optical thickness of the rings is about unity[10], then the probability of an atom striking a ring particle is $\approx(1-e^{-1})=0.63$. For most of these collisions, the atom will be reflected, but roughly one impact in five will result in loss of an atom. For a nominal keplerian orbital period of $T=4\times10^4$ s, the resulting lifetime is $\tau=T/(0.63\times0.22)=3\times10^5$ s. With this lifetime, the source rate Q required to satisfy the observations is estimated to be $Q=N/\tau=8\times10^{33}/3\times10^5=3\times10^{28}$ atom s^{-1}. Note that this derived source rate is insensitive to the actual trajectories. If the orbits extend beyond the field-of-view of the rocket aperture (which was used for the abundance estimate) then the fraction of time that an atom spends within the field is less than unity, and the orbital period T is also greater. As both the total abundance and lifetime estimates are increased, then the derived source rate is approximately the same.

The first investigations of H atom production by the rings were presented by Blamont[4] and Dennefeld[5], who considered several sources, including sublimation and dissociation of ring material, and bombardment by meteoroids, the solar wind, and interstellar gas. Their calculated source rates are summarised in Table 1 and are evidently too small to account for the required value of 3×10^{28} atom s^{-1}. Cheng and Lanzerotti[6] proposed sputtering by high energy ions as a ring erosion mechanism. Assuming that peak jovian fluxes can be used to describe the saturnian magnetosphere, they predicted a hydrogen atom source rate $\approx1.2\times10^{27}$ s^{-1}. However, the Pioneer 11 flyby has shown that the Saturn magnetosphere is much less intense than that of Jupiter, and the peak fluxes used by Cheng and Lanzerotti should be reduced by a factor of at least 20, and probably more, based on measured saturnian proton and electron fluxes[11,12]. Furthermore, proton fluxes[12] in the vicinity of the rings are $<10^{-2}$ of the peak values, which results in an ion-sputtering rate of $<6\times10^{23}$ atom s^{-1}. An additional mechanism has been proposed by Ip[7]—neutralisation by ring particles of the photoelectron–ion flux flowing out from the Saturn ionosphere along magnetic field lines. The magnitude of this source is also insufficient to account for the observations (see Table 1).

Table 1 Ring H atom production rates

Mechanism	Q (atom s^{-1})
Photo-sputtering	1.5×10^{28}
Photoelectron–ion neutralisation	9×10^{23}–9×10^{24}
Sublimation, dissociation	2.2×10^{24}
Meteoritic bombardment	1.8×10^{24}
Magnetospheric ion sputtering	$<6\times10^{23}$
Solar wind	1×10^{23}
Interstellar gas	2.2×10^{22}

Harrison and Schoen[13] pointed out that UV radiation ($1{,}000\leqslant\lambda\leqslant1{,}800$ Å) will dissociate water molecules on ice surfaces and emit hydrogen atoms and hydroxyl radicals. Similar processes have been found to occur on UV irradiation of glasses and semiconductors[14,15]. For photo-absorption which occurs in the outer few molecular layers of an ice crystal, the probability of escape from the crystal is high. At the orbit of Saturn, the molecular photodissociation rate[16] due to solar UV radiation is 1.1×10^{-7} s^{-1} molecule^{-1}. If we assume that only the single outermost monolayer of molecules (1×10^{15} molecule cm^{-2}) of the icy ring material will contribute to the flux escaping from the solid, then we find a hydrogen atom flux of 1.1×10^8 atom cm^{-2} s^{-1}. Although the initial energy distribution cannot be accurately determined, most of these atoms should possess less than the 3 eV of energy required for escape from the Saturn system, due to simultaneous vibrational and electronic excitation of OH, collisions with other surface molecules, and subsequent collisions with ring particles.

The projected area of the A + B rings at the time of the rocket measurements was 1.4×10^{20} cm^2, resulting in a total source rate of $Q=1.5\times10^{28}$ atom s^{-1}. This value is compared with other mechanisms in Table 1.

The source rate for the mechanism proposed here is within a factor of two of the required value and is within the experimental error of the measurement. Considering the spirit of the calculations, the agreement is quite satisfactory, arguing that the proposed mechanism is a viable explanation for the observed Lyα glow. It may be possible to test this, and other, source hypotheses through the recent Pioneer 11 and forthcoming Voyager flybys. For the present mechanism, the source strength during these flybys will be smaller due to the smaller inclination of the ring plane relative to the Sun.

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Cooling-rate dependence of thermoremanent magnetisation

J. M. W. Fox & M. J. Aitken

Research Laboratory for Archaeology,
Oxford University, 6 Keble Road, Oxford, UK

In using the thermoremanent magnetisation (TRM) of material heated in antiquity (usually pottery, bricks, stones or lava) to evaluate the magnitude of the geomagnetic field at the time of heating, little attention (apart from an observation by Thellier[1]) has been given to the possibility that results are affected by the rate at which the sample is cooling while the TRM is being acquired. We report here an experimental observation of the effect in archaeological baked clay; it parallels the theoretical prediction of Dodson and McClelland-Brown[2] in respect of geological material.

The effect was first noted during a calibration experiment in which samples of Peruvian pottery[3] were refired to 720 °C in a known laboratory field. The TRM acquired during the 7-h cooling from this refiring was then analysed routinely using the zero-field variant of the Thellier[4] method. In this the cooling-times were of the order of 30 min. The samples were in the form of disks, (diameter 25 mm; thickness 8 mm) and before the experiment they had been thermally stabilised by prolonged baking. Magnetisations emplaced during the analysis were in the same direction with respect to the sample as the magnetisation being analysed; hence any effects due to sample shape or fabric anisotropy[5] were avoided. The samples were measured on a Digico fluxgate archaeomagnetic-head magnetometer.

The results are given in Table 1, for both the Peruvian pottery and for samples fabricated from Oxfordshire clay. The field magnitude evaluated by the Thellier analysis is higher than the known field in which the samples had cooled during acquisition of the TRM being analysed, on average by 6% and 12% respectively. The values for the known field were obtained with a fluxgate magnetometer which had been calibrated against a proton magnetometer. The fields used in the course of the analysis were within 0.5% of 48.3 μT.

Table 1 Evaluation by TRM of field intensity used for refiring

Specimen no.	Specific magnetisation ($\times 10^{-3}$ A m^2 kg^{-1})	F^{A}* (μT)	
P12P01P	2.5	52±1	Av. 53
Q	2.4	53±1	
Y	2.5	54±2	
Z	2.5	54±1	
P43C01T	4.6	51±1	Av. 53
U	3.3	55±1	
Y	4.8	50±3	
Z	3.7	55±1	
Islip 02	4.3	54±2	Av. 54
03	5.5	54±1	
04	3.4	53±1	

The cooling time after refiring was 7 h. The cooling times during TRM analysis were ~30 min. For the Peruvian sherds, P12P01 and P43C01, the field intensity used for refiring was 50 μT; for the Islip specimens it was within 0.2 μT of 48.3 μT. F^A is the value deduced for the refiring using Thellier zero-field analysis of the TRM acquired during cooling down from refiring.

* ±1 s.d.

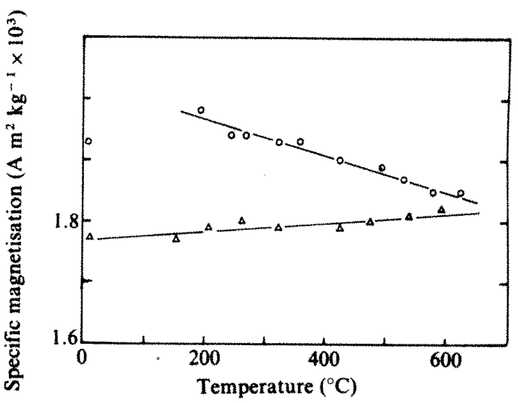

Fig. 1 Remagnetisation of Peruvian pottery sample (P12P10Y) following refiring to 720 °C. The TRM was measured (at room temperature) after remagnetisation during cooling from various reheating temperatures. Two sets of remagnetisations are shown: the first (○) followed a refiring in which the cooling time was 7 h, the second (△) followed a refiring in which the cooling time was half an hour. The latter was also the maximum cooling time for the remagnetisations. The magnetic field was 48 μT in all cases.

In the above analytical procedure, at each temperature step the sample cools in zero field before being remagnetised. To avoid possible interference by a memory effect the following more direct experiment was carried out. After refiring to 720 °C, either with a cooling time of 7 h or of 30 min, the sample was reheated to successively increasing temperatures and cooled rapidly, in ~30 min, the reheating and cooling being in the same field as for the 720 °C refiring. After each step the magnetisation of the sample was measured (at room temperature); a typical result is shown in Fig. 1. Whereas for the 30-min cooling the sample shows a slight overall increase, by <1%, as the reheating steps proceed; for the 7-h cooling there is a decrease, by ~8%.

Figure 2 shows the results of a similar experiment carried out using the SQUID magnetometer (and its associated oven) developed for archaeomagnetic work by Walton[6]. In this the sample (a small 3-mm diameter by 3-mm thick core of Greek pottery) does not have to be demounted from its quartz presentation rod between in-field heating and measurement (at room temperature). After rapid cooling from 600 °C, in a few minutes,

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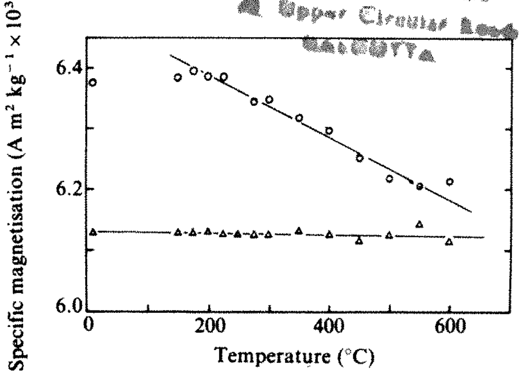

Fig. 2 Remagnetisation of Mycenaean pottery sample (1632a6) following refiring to 600 °C. The TRM was measured (at room temperature) after remagnetisation on cooling from various reheating temperatures. Two sets of remagnetisations are shown: the first (○) followed a refiring in which the cooling time was 2.5 h, the second (△) followed a refiring in which the cooling time was a few minutes. The latter was also the maximum cooling time for the remagnetisations. The magnetic field was 74 μT in all cases.

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Table 2 Effect of different cooling times on TRM evaluation of field intensity used for refiring

Sample reference	Overestimate relative to 5-min cooling times	
	For 2-h (%) cooling time	For 16-h cooling time
32a6.1 (Attic)	2	7
35a1.1 (Late Minoan)	7	11
P18 L03 (Peruvian)	9	14

TRM evaluation was by means of the Thellier method, using a SQUID magnetometer. Refiring was done in a small non-inductively wound oven external to the magnetometer which was lowered over the sample. For the 5-min cooling time the oven was lifted off the sample; for the 2-h cooling time it was left in position with the heater current switched off; for the 16-h cooling time the heater current was turned down at a uniform rate (by means of a motor driven Variac). For each sample a 5-min cooling time run was done initially and finally. The TRM evaluations from these pairs agreed to within ±1%.

reheating and cooling (also in a few minutes) in the same field produced no change in magnetisation as successively increasing temperatures were used for the reheating. On the other hand, when the sample was cooled slowly from 600 °C, in 2.5 h, the subsequent magnetisation was 5% higher than for quick cooling, and as the reheating steps proceeded (each followed by quick cooling) the excess disappeared. For the slow cooling the heater current for the oven was turned down manually. The oven was non-inductively wound and the a.c. field at the sample site was <20 μT. In the large-sample experiments there was no current in the oven for the slow cooling, the fast cooling being achieved by blowing air on the samples after lifting off the oven.

The effect has also been observed[7] with other samples (see Table 2); in all cases the TRM acquired for slow cooling is greater than for fast cooling. This is as Dodson and McClelland-Brown[2] predicted in considering samples in which the magnetic carriers are monodomain grains of haematite and magnetite. The basis of their prediction is that for a given group of grains the effective blocking temperature is lower in the case of slow cooling and therefore the fractional alignment that is blocked-in is higher, both because of the reduced thermal agitation at the lower temperature and because of the intrinsic increase of activation energy. Dodson and McClelland-Brown[2] consider very slow, geologically-relevant, cooling rates, in the range 6 °C min^{-1} to 3 °C Myr^{-1}; they predict a 7% increase in TRM for an order of magnitude decrease in cooling rate. Our results accord with this prediction. The magnitude of the effect will depend on the magnetic mineralogy of the sample and hence it is not to be expected that it will be quantitatively the same for all samples. This aspect is under investigation.

In general, archaeological pottery and bricks fired in kilns will have cooled down after a couple of days[8]. The cooling times used routinely in this Laboratory for Thellier analysis are either 30 min or a few minutes. Hence the magnitude of the ancient field so evaluated may be larger than the true value by 10–20%; for laboratories employing longer cooling times, or for samples that cooled quickly after the archaeological firing (for example, in an open hearth or in a clamp kiln), the effect will be less. Clearly it would be desirable to investigate the strength of the effect in each type of sample and to make an appropriate correction, or to use laboratory cooling times of the same order as the presumed archaeological cooling time.

The significance for existing determinations of ancient intensity is that they are also liable to be overestimates. This will be significant when calculating the global radiocarbon content. If, for instance, the magnitude of the geomagnetic moment was in general 10% less than indicated by the global curve of Cox[9], the historical radiocarbon production rate will have been ~5% more than currently assumed.

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Bubbles and breaking waves

S. A. Thorpe

Institute of Oceanographic Sciences, Wormley, Godalming, Surrey, UK

P. N. Humphries

Department of Physics, Surrey University, Guildford, UK

The physical processes which control the transfer of gases between the atmosphere and oceans or lakes are poorly understood. Clouds of micro-bubbles have been detected below the surface of Loch Ness when the wind is strong enough to cause the waves to break. The rate of transfer of gas into solution from these bubbles is estimated to be significant if repeated on a global scale. We present here further evidence that the bubbles are caused by breaking waves, and discuss the relationship between the mean frequency of wave breaking at a fixed point and the average distance between breaking waves, as might be estimated from an aerial photograph.

It has been suggested[1] that there may be a causal relationship between whitecaps, or breaking waves, on the surface of a freshwater lake and the individual clouds or bubbles which have been detected acoustically below the surface. The clouds appear with a typical frequency of about 1 per min in wind speeds of 10 m s^{-1} and penetrate to depths of at least 5–10 m. To investigate the relationship, and other phenomena, we measured breaking waves in Loch Ness using a capacitance wire probe[2] and time-lapse photography.

The capacitance wire probe was placed on a mooring connected to shore[3] in a water depth of ~160 m and 250 m from the shore, where the fetch for north-east or south-west winds is some 10 km. The wind speed was measured by an anemometer on a tripod in the loch. As the slope of the water surface in a breaking wave is large, the electronic circuit used with the probe was designed to detect large gradients in surface height. The signal voltage from the probe, proportional to the wave height, was passed through a differentiator and the output compared with a reference level which could be set according to the wave conditions. When the differentiated signal exceeded the reference, a spike generator was triggered which marked the record.

The slope, $\partial h/\partial x$, of the water surface is related to the time derivative, $\partial h/\partial t$, of the wave height measured at a point, by

$$\frac{\partial h}{\partial t} = c\frac{\partial h}{\partial x} \tag{1}$$

where c is the phase speed of the waves. For small deep-water waves $c = c_0 = gT/2\pi$, where T is the wave period and g the acceleration due to gravity[4]. For large amplitude waves the

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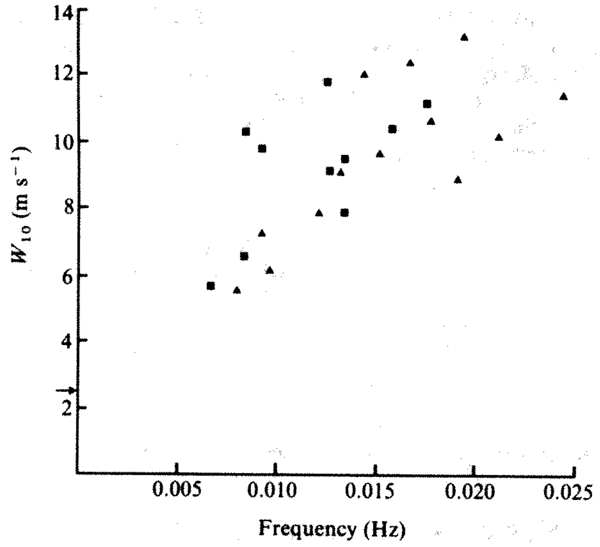

Fig. 1 Wind speed, W_{10} (corrected to 10 m) versus frequency f, of bubble clouds seen on a sonar record[1] (■), or frequency of breaking waves (▲), only one wave being counted when neighbouring waves break as the sonar record cannot resolve a time scale corresponding to individual waves, the bubble clouds from neighbouring waves are expected to merge. Observation periods of at least 20 min were used. The mean standard deviation of the number of waves in a five minute period is ~2.6. The arrow marks the approximate wind speed at which whitecaps and bubbles are first observed.

phase speed is a function of the wave slope. The maximum slope of a regular progressive wave train in deep water[5] is close to 30° when the phase speed[6], $c = 1.0923c_0$, and hence the maximum value of the time derivative is approximately

$$\frac{\partial h}{\partial t} = 0.1004gT \qquad (2)$$

(The greatest wave speed is estimated[6] at $c = 1.09295c_0$ and hence equation (1), with $c = c_0$, may be used with an error of <10%).

The capacitance wire probe was used to measure the mean wave period, T, estimated from the zero-crossing interval, and equation (2) used to estimate $\partial h/\partial t$ for a limiting wave. In practice the reference level setting was set empirically to trigger 'spikes' on an ink-plotting pen when a breaking wave could be seen at the probe, and considerably smaller values than the theoretical equation (2) were found to be suitable. Trouble was encountered by spikes being triggered near wave nodes where the wave slope is large but where no breaking or bubble entrainment occurs. Waves tend to break near their crests and these spurious spikes were avoided by incorporating a circuit which passed the signal spike only if the wave height exceeded a certain level, which was set in relation to the observed height of the existing wave field.

The success of the system in identifying breaking waves was tested by taking cine film of the probe and water surface from shore and by comparing the record of wave height and spikes with the photographic record. In calm weather tests were also made by artificially creating breaking waves in the wake of a boat which passed close by the probe. The detection system was insensitive to brief spikes of the order of 25 ms or less in which $\partial h/\partial t$ just exceeds the threshold. Waves less than the probe dimensions, ~10 cm, were not resolved either by the probe or by the camera, but larger waves with crest lengths exceeding this dimension were detected.

For wind-waves Loch Ness is effectively an enclosed basin, and swell from distant storms which might, at sea, induce wave breaking[7], is absent. A relationship might therefore be expected between the frequency of wave breaking and the local wind speed. Figure 1 shows the average frequency of wave-breaking events and individual bubble clouds seen in the sonar records[1], plotted against wind speed. There is considerable scatter, arising partly from the uncertainty in identifying individual bubble clouds in the sonar records and partly from the unsteadiness of the wind. There is an upward trend in both sets of points, commensurate with a zero value at a wind speed of ~2.5 m s^{-1} when whitecaps and bubble clouds are first observed. Whilst the frequencies are similar, the number of wave-breaking events exceeds that of the bubble clouds. A contributing factor may be that some waves, breaking 'gently', do not produce bubbles which penetrate below the depth of the pre-existing cloud.

The average period between the occurrence of breaking waves at the probe was compared with the mean distance between breaking waves approaching the probe. The latter was derived from time-lapse photographs of the water surface taken from the hillside above the loch. The width of the camera frame was established by taking photographs of a rubber boat of known dimensions. The time interval between frames was such that groups of waves would travel a distance well exceeding the frame width during the time interval, and thus be filmed only once. With the assumption that their statistics were uniform, the mean distance between whitecaps, $\bar{x}$, was found by dividing the number of breaking waves observed to intersect a line along the loch axis (approximately the mean direction of wave advance) into the total effective frame length.

The large and steep waves which break, forming whitecaps, occur in groups. It was very rare to see two neighbouring waves break simultaneously and when this happened only one wave was included in the count. Figure 2 shows estimates of $\lambda/\bar{x}$, where λ is the mean wavelength estimated from using the relation[4] $\lambda = gT^2/2\pi$. Also shown are estimates of $\bar{t}/T$, where $\bar{t}$ is the average time interval, measured by the capacitance wire probe, between successive groups which contained breaking waves. About 58% of the groups which passed the probe with breaking waves contained more than one (none more than three). There is again much scatter in the observations (again variation in wind conditions may have contributed) but evidence of an increase in both $\lambda/\bar{x}$ and $T/\bar{t}$ with wind speed. This is partly due to an increase in T (and hence λ) with wind speed from an average of 2.58 s at wind speeds of 6.17 m s^{-1} to 3.05 s at winds of 12.4 m s^{-1}. On average the values of $\lambda/\bar{x}$ are significantly greater than those of $T/\bar{t}$ at the same wind speeds. (Mean values are 0.076 and 0.056 respectively at a mean wind speed of 9.9 m s^{-1}).

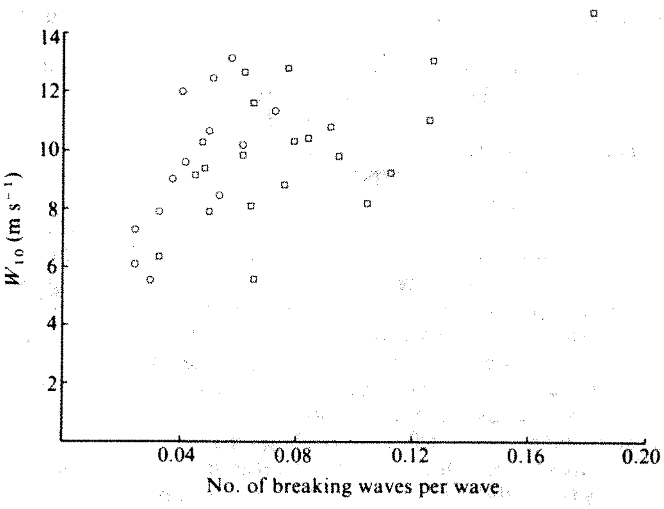

Fig. 2 Wind speed, W_{10} (corrected to 10 m) versus ○, $T/\bar{t}$ or □, $\lambda/\bar{x}$, the number of breaking waves per wave, groups counting once only (see text). $\lambda/\bar{x}$ was calculated over an effective horizontal length of at least 2,280 m.

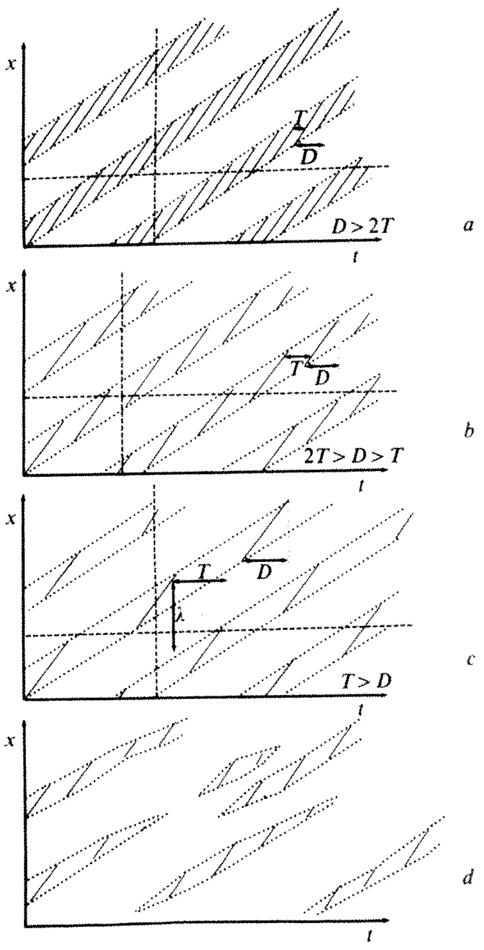

Fig. 3 Space (x)–time (t) diagram of the movement of breaking waves and groups. The heavy lines are breaking waves and the dotted lines are groups. Idealised diagrams when: *a*, $D>2T$; *b*, $2T>D>T$; *c*, $T>D$; *d* is a sketch of a more realistic situation in which the groups break intermittently. The groups of breaking waves have been drawn closer together than they are in reality. The dashed lines x, constant; and t, constant indicate the way in which the breaking waves are sampled by the probe and by photographs respectively. In *a* these lines cut at least one breaking wave in each group. In *b*, t, constant misses, but x, constant always intersects, one or two of the breaking wave tracks, whilst in *c* both lines occasionally miss and more than one breaking wave in the same group is never sampled.

The regions of large waves, and hence whitecaps, advance at the group velocity[8], which, in deep water, is half the phase speed of the waves. Figure 3*a*,*b*,*c* shows the waves in space–time coordinates. The individual breaking waves, advancing at the phase speed, are shown as solid lines of slope $c=\lambda/T$. The groups containing breaking waves advance at the group velocity and are indicated by a succession of these lines, forming a patch outlined by dotted lines with slope $c/2$. If the duration of persistance of a whitecap, D, is more than double the wave period (Fig. 3*a*) each group with breaking waves will contain at least one breaking wave when it is observed at constant time (by a photograph) or as it passes a constant position (the wave probe). If only one breaking wave in each group is counted, the ratio r of the mean distance between such successive observations at constant time and their mean period will be the group velocity, $c/2$. Hence the ratio

$$\chi \equiv (\lambda/\bar{x})/(T/\bar{t}) = \frac{c}{r} = 2 \qquad (3)$$

If, however, $2T>D>T$ (Fig. 3*b*), whilst each group containing breakers will be recorded by at least one breaking wave at the probe (constant x), there are times when no waves at all will be breaking in a particular group, and hence $\chi<2$. Moreover at most one wave at a time will be breaking in a group. If, as is often observed, $D<T$, then groups containing breaking waves may pass the probe with no breaking event registering. Groups containing multiple breaking waves are more likely to be recorded at a fixed location than to be seen in a photograph, as is indeed observed. The occurrence of multiple breaking waves at the probe suggests that D frequently exceeds T, whilst their absence in photographs implies that D is usually less than $2T$.

The observed mean value, $\chi=1.65$, is consistent with this argument. Because the breaking waves are unlike the idealised model (but resemble Fig. 3*d*) there seems at present no obvious way in which χ may be related to the simple statistics of the wave field. A working value of χ would, however, be useful as it would then be possible, by finding $\bar{x}$ and T (or λ)[8] from aerial photographs, to estimate $\bar{t}$ and hence to determine how often a stationary structure, for example, an oil rig or wave power device, would be subject to groups of breaking waves.

Whilst there is at least circumstantial evidence for a causal relationship between breaking waves and bubbles, more research is needed. Progress might be made by using narrow-beam upward-pointing sonar which could resolve details of the wave surface during wave breaking.

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Gallium porphyrins in bituminous coal

Raymond Bonnett & Franciszek Czechowski

Department of Chemistry, Queen Mary College, Mile End Road, London E1, UK

Metalloporphyrins are minor constituents in crude oil and its relatives[1] but their presence in coal is not clearly established. This is surprising as such complexes are thought to arise from chlorophyll, and coal is largely of plant origin. Although coal samples were among the earliest examined in this context[2,3], most later workers[1,4] followed Treibs[5] in mainly considering crude oils, asphaltenes and shales. Weigelt and Noack[2] presented early evidence for chlorophyll derivatives in fossilised leaves from brown coal, while Treibs[3] found trace amounts of metal-free porphyrins in various coals. Uspenskii and Gorskaja[6] confirmed the latter finding, but observed that the porphyrin content of sapropelic coals such as cannel coal was much higher than that of hard coals. Metalloporphyrins in fossil plant material are important because they provide information on the geochemical transformations of chlorophyll. Moreover, metal complexes with organic ligands (such as the metalloporphyrins) are of particular interest in contemporary coal chemistry because they are probably extracted by the organic solvent in the conditions used in certain coal liquefaction processes, and hence may modify the activity of catalysts used in subsequent chemical reactions with the coal extract. We report here the isolation of a metalloporphyrin fraction from a British bituminous coal, and show that the principal metal present in the isolated material is gallium.

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Table 1 Metal analysis of metalloporphyrin fraction isolated from Daw Mill coal

Metal	%
Gallium	5.7
Sodium	0.49
Iron	0.36
Potassium	<0.2
Magnesium	<0.2
Aluminium	0.17

Powdered dried coal (92 kg) from Daw Mill Colliery (South Midlands, Int. Code No. 811) was extracted with 7% v/v sulphuric acid in methanol for 12 h. The filtrate was diluted and much of the organic material was transferred into chloroform, which was washed (aqueous $NaHCO_3$, water) and taken to dryness (42.8 g). This crude extract was submitted to column chromatography on silica gel, the elutriant being benzene–methanol. Porphyrin-containing fractions were combined and submitted to preparative TLC on silica gel irrigated with benzene–ammoniacal methanol. Three red bands, barely separated, appeared at $R_F \sim 0.5$–0.55. These were combined, extracted and submitted to further purification by TLC. Finally the orange-red material was extracted with dichloromethane. The extract was filtered, concentrated and treated with petroleum ether to give the metalloporphyrin concentrate (17.8 mg, representing 0.19 p.p.m. of coal).

The metalloporphyrin concentrate thus obtained was a purple solid, not obviously crystalline but possessing a metallic sheen, and it did not melt below 300 °C. Metal analysis by thermal neutron activation (AERE, Harwell) showed that gallium was the principal metal present, with much smaller amounts of sodium, iron, potassium, magnesium and aluminium (Table 1). These latter metals possibly represent inorganic impurities, although iron and aluminium may both be present as metalloporphyrins.

A solution of the metalloporphyrin fraction in chloroform was red, and had a brilliant orange-red fluorescence in UV light (365 nm). The absorption spectrum (Fig. 1), with a Soret band at 405 nm, and visible peaks at 532 and 570 nm was typical of a metalloporphyrin, and resembled that of hydroxogallium(III) octaethylporphyrin ($\lambda_{max}^{CHCl_3}$ 404, 533 and 571 nm). The NMR spectrum accorded with that expected for a polyalkylporphyrin complex of a diamagnetic ion. The signals were broad, however, and this is attributed to the presence of a mixture of such complexes and to the effect of small amounts of paramagnetic ions.

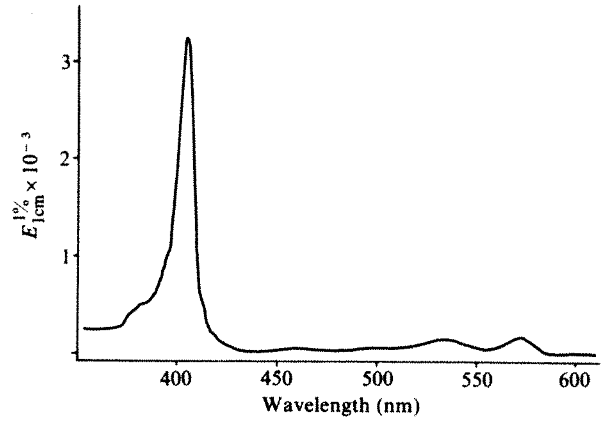

Fig. 1 Absorption spectrum of the metalloporphyrin fraction from Daw Mill coal. Solvent, chloroform.

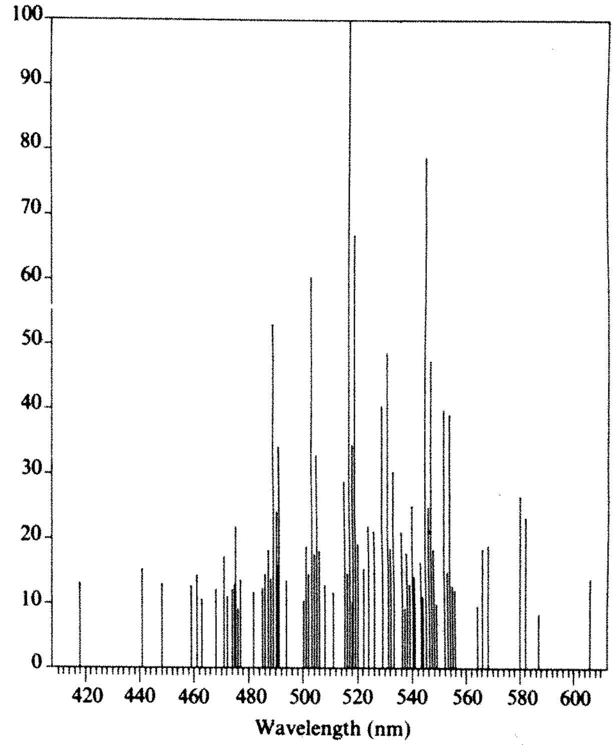

Fig. 2 Mass spectrum of metalloporphyrin fraction from Daw Mill coal. The sample was introduced with a direct insertion probe, with a source temperature of 300 °C (measured at PCMU, Aldermaston).

The mass spectrum (Fig. 2) was very informative, and clearly indicated a mixture of homologous metalloporphyrins. The largest peak, *m/e* 517.185, corresponded to $C_{30}H_{32}N_4\,^{69}Ga$ (the axial ligand having been lost), and was attended (natural distribution[8] of gallium isotopes $^{69}Ga:^{71}Ga = 60.4:39.6$) by a second peak at *m/e* 519.188 ($C_{30}H_{32}N_4\,^{71}Ga$). These molecular formulae correspond to complexes of didemethyletioporphyrin, or an isomer of this. Some other components of this homologous series are given in Table 2, from which it is seen that the second most abundant pair of ions (*m/e* 547, 545) correspond formally to the gallium complex of tetraethyltetramethylporphyrin (etioporphyrin). Although each ion may well represent an isomeric mixture, the formulations suggested above are favoured on geobiogenetic grounds and the general analogy with the petroporphyrins[1]. Support came from HPLC (Fig. 3) using a reverse phase system[7] which proves to be very useful in the analysis and separation of polar porphyrins such as the gallium complexes. This revealed several components, one of which had the same retention time, within the resolution of the column, as synthetic hydroxogallium(III) etioporphyrin I.

Small-scale experiments on other coals gave similar but not identical results. Three other British bituminous coals (Annesley, South Nottinghamshire, Int. Code No. 633; Bersham, Western Region, Int. Code No. 634; and Linby, South Nottinghamshire, Int. Code No. 732) contained metalloporphyrins with similar properties, as did a Turkish lignite (Ankara-Beypazari). However, samples of a German brown coal and a Polish brown coal (Konin) contained a porphyrin fraction with different properties. With the amounts of porphyrin detected (<0.6 p.p.m.) it is important to appreciate the dangers of contamination, especially in a laboratory where porphyrins have been studied for many years. The different results obtained with the brown coal samples argue against this possibility, and it turns out that gallium porphyrins had not been prepared in our laboratory before this work. Moreover the gallium complex of octaethylporphyrin (a common ligand in our laboratory) was not present in the metalloporphyrin concentrate from coal (its

Table 2 Some accurately measured ions in the mass spectrum of the metalloporphyrin fraction from Daw Mill coal

Observed mass	Abundance	Formula	Calculated mass
547.220	47.3	$C_{32}H_{36}N_4{}^{71}Ga$	547.219
545.218	78.7	$C_{32}H_{36}N_4{}^{69}Ga$	545.220
533.205	30.3	$C_{31}H_{34}N_4{}^{71}Ga$	533.203
531.200	23.0	$C_{31}H_{34}N_4{}^{69}Ga$	531.204
519.188	66.7	$C_{30}H_{32}N_4{}^{71}Ga$	519.188
517.185	100	$C_{30}H_{32}N_4{}^{69}Ga$	517.188
505.171	32.8	$C_{29}H_{30}N_4{}^{71}Ga$	505.172
503.169	60.2	$C_{29}H_{30}N_4{}^{69}Ga$	503.173
491.157	34.0	$C_{28}H_{28}N_4{}^{71}Ga$	491.156
489.155	53.0	$C_{28}H_{28}N_4{}^{69}Ga$	489.157
477.140	13.6	$C_{27}H_{26}N_4{}^{71}Ga$	477.140
475.137	21.7	$C_{27}H_{26}N_4{}^{69}Ga$	475.141

Measured at PCMU, Aldermaston, on a Kratos (AEI) MS50 mass spectrometer with a DS-50SM data system. Direct insertion, 300 °C at 70 eV.

presence would have been revealed by a pair of peaks m/e 601 and 603 in Fig. 2).

Contamination with gallium is perhaps less likely. Nevertheless, we have felt it necessary to carry out control experiments in which the chromatographic materials were treated in appropriate conditions with trace quantities of octaethylporphyrin. The latter substance was recovered, but the gallium complex was not detected. Nor was gallium incorporated when basic gallium acetate was treated with octaethylporphyrin in 7% H_2SO_4–MeOH (18 h at room temperature, that is approximating to the conditions of the coal extraction). This experiment is important, as gallium is one of several trace metals present in coal[9,10]: indeed, processes are described for the isolation of the metal from this source[11].

Because of the possibility of fractionation during the coal extraction, we do not consider that we have isolated all the metalloporphyrins present in the coal sample, and clearly several minor peaks in the mass spectrum remain to be assigned. These will be discussed elsewhere. The mass spectrum does suggest, however, that the ratio between gallium complexes of the etioporphyrin series ($M \pm 14n$, $M = 545$, 547) and the analogous complexes of the presumed 13^2,15-cycloetioporphyrin III (deoxophylloerythroetioporphyrin) series ($M' \pm 14n$, $M' = 543$, 545) is generally high. This, and the low abundance of the metalloporphyrins in bituminous coal compared with that in many crude oil deposits, is consistent[12] with the more drastic thermal conditions believed to be necessary for coal to form. Indeed, it seems reasonable to suppose that coal rank may be inversely related to available metalloporphyrin content.

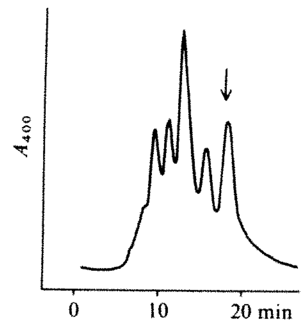

Fig. 3 HPLC of metalloporphyrin fraction on reverse phase column (Waters, Bondapak C_{18}/Porasil B) eluted with MeOH:H_2O = 72.5:27.5. The arrow indicates the component with a retention time indistinguishable from that of synthetic hydroxogallium(III) etioporphyrin. The column resolves homologues, not isomers, of polyalkylporphyrins.

The presence of gallium in coal porphyrins is unexpected: the abundance of gallium in the Earth's crust (15 p.p.m.) is an order of magnitude less than that of vanadium[8], and, although some organisms accumulate gallium[13], the biological function of this does not seem to be understood. The vanadyl porphyrins which occur in crude oil are thermodynamically and kinetically stable, and it may be argued that once formed they persist through geological time. Whether a similar argument can be applied to gallium porphyrins is not known: they are chemical rarities and require further study.

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No-flow equilibration and adsorption dynamics during ionic transport in soils

V. Murali & L. A. G. Aylmore

Department of Soil Science and Plant Nutrition, University of Western Australia, Nedlands, Western Australia 6009, Australia

Methods for predicting the movement of adsorbing chemicals such as fertilisers, pesticides, herbicides and heavy metals in soils have received increasing attention because of their importance in relation to the efficiency of fertiliser usage and in the control of agricultural and industrial pollutants[1]. Because of the difficulty in coping with the complex non-linear partial differential equations necessary to describe the dispersive–convective–adsorptive flow process, analytical solutions have been largely limited to the assumptions of instantaneous equilibrium and simple linear adsorption isotherms[2–5]. We demonstrate here the need to incorporate adsorption dynamics to describe adequately practical situations, particularly those involving intermittent flow.

In reality the movement of water through soils rarely, if ever, occurs continuously or at uniform flow velocity for any length of time. For example, the water content of the soil described as 'field capacity'[6] results from the much slower redistribution of water following infiltration. Consequently there is a period following infiltration when flow is negligible but adsorption/desorption processes may continue. Thus, rather than being able to assume a constant or average pore water velocity and instantaneous equilibration between solute and adsorbent, it is imperative to consider the effects of the 'go–stop' nature of water movement where adsorption dynamics are concerned.

The importance of this effect is illustrated by our recent studies of the influence of adsorption dynamics on the movement of adsorbing ions in soil columns. The term 'adsorption dynamics' is used here to include not only adsorption kinetics at

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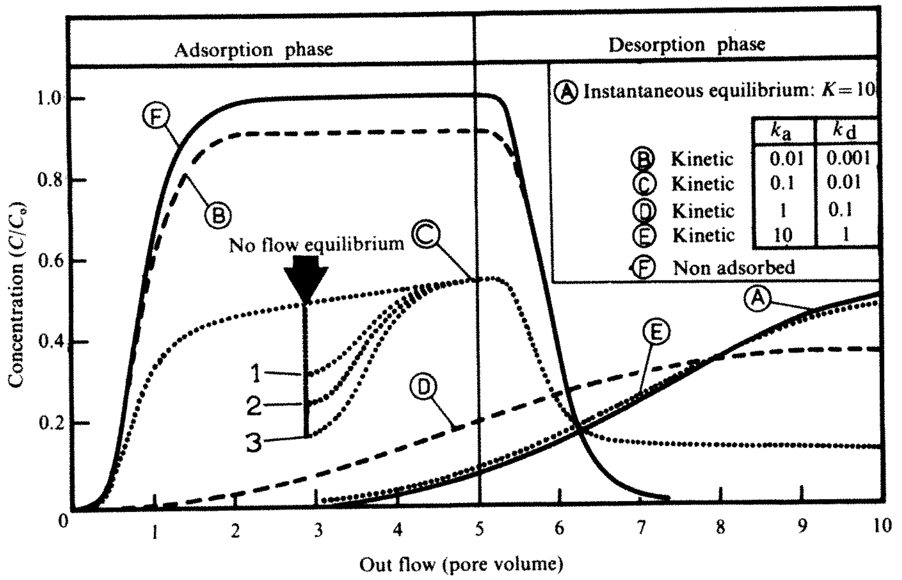

Fig. 1 Simulation of adsorption–desorption breakthrough curves for instantaneous equilibrium (curve A), kinetic models (B, C, D, E) and non-interacting solute (F). Effects of no-flow equilibrations are shown on curve C for increasing periods equivalent to the time taken for the outflow of 2, 4 and 10 pore volumes respectively.

solid surfaces, but also any intermediate steps which may influence the rate at which chemicals are exchanged between solution and adsorbed phases. In particular this includes diffusion from the centres to the walls of hydrodynamic pores (that is, pores responsible for hydrodynamic dispersion) but excludes intra-aggregate diffusion which can be viewed as a separate process.

A standard technique for studying the movement of ions in soil[7,8] is the measurement of 'breakthrough curves' which describe the change in the outgoing solution from a soil column with outflow volume following a change in input concentration. Figure 1 contrasts the breakthrough curves, predicted by computer simulations using numerical methods, for non-instantaneous equilibration involving a range of different adsorption and desorption rates (k_a and k_d respectively), with that obtained for instantaneous equilibration (at the same equilibrium constant) using a simple linear isotherm. The simulations depict the passage of five pore volumes of solution containing the ion into a column of soil saturated with water (adsorption phase) followed by five pore volumes of ion free water (desorption phase). The value of the equilibrium constant K at relevant soil solution concentrations is generally greater than unity and $K = 10$ has been used as a suitable example.

The assumption of instantaneous equilibrium means that for two ionic species having the same or similar equilibrium isotherms, identically shaped and positioned breakthrough curves (curve A) will be predicted for the passage of their concentration pulses through a soil column. However, the rates of approach to adsorption and desorption equilibrium may vary considerably between different species and this will result in a range of markedly different breakthrough curves depending on the relevant rate coefficients (for example, curves B, C, D and E).

The consequence of different 'no-flow equilibration' periods on the breakthrough curves predicted in any case involving adsorption dynamics is particularly relevant. This is illustrated by the depressions in outgoing concentration for curve C corresponding to no-flow periods of increasing length. The assumption of instantaneous equilibration (curve A) will, of course, obscure this effect completely.

In the previous simulations simple linear adsorption kinetics were used to demonstrate the effects of time dependency of the adsorption/desorption processes. However, in other simulations we have shown that the same considerations are relevant regardless of the type of isotherm involved, for example, Freundlich and Langmuir.

The considerable practical significance of this effect is demonstrated by the breakthrough curves in Fig. 2 obtained for solutions of phosphate (2.5 mM), selenite (1.0 mM) and sulphate (1.0 mM) ions, and tritium labelled water including a 24 h no-flow equilibration period. The soils used were in a finely-ground state and the depressions in outgoing concentration for phosphate and selenite following no-flow periods cannot be attributed to intra-aggregate diffusion. This is verified by the absence of measurable depressions in the case of the sulphate despite the measurable adsorption of this ion and for the effectively non-adsorbed tritium ion. Thus it is clear that adsorption dynamics as previously defined, can be particularly important in controlling ionic transport in real conditions. The significant difference in the shapes of the breakthrough curves for sulphate and tritium, despite the absence of no-flow depressions for both, clearly demonstrates that adsorption kinetics form only part of the total adsorption dynamics influencing ionic movement. Other studies demonstrate that in aggregated systems the involvement of intra-aggregate diffusion before surface interactions, introduces no-flow depressions in outgoing concentrations for even non-adsorbed species and greatly enhances those for adsorbing species.

Such intermittent flow/no-flow measurements apparently provide a simple and convenient technique for checking the pertinence of adsorption dynamics for any particular ion–soil system, solution concentration and flow velocity, and we are not aware of any previous such demonstration in non-aggregated systems.

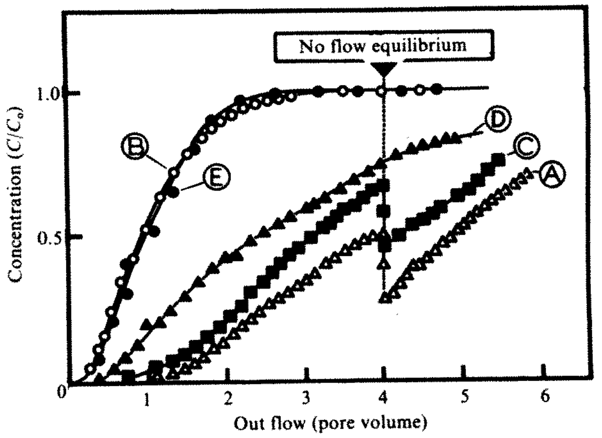

Fig. 2 Experimental breakthrough curves for phosphate (A) and tritium (B) movement through Yalanbee sandy loam and selenite (C), sulphate (D) and tritium (E) through York sandy loam showing effects of 24-h no-flow equilibration. Flow velocity equivalent to 0.3 pore volumes per hour.

The nature of the rate determining steps in these studies will require further investigation. While studies on equilibrium distributions involving agitation and high solution-to-solid ratios generally indicate extremely fast ion–soil interactions[9], the present observations demonstrate that such measurements of adsorption kinetics may be of little relevance in relation to ionic transport. There is increasing evidence that considerably slower rates are involved in actual soil systems[10,11].

These observations form part of a much more detailed study of ionic transport through soil systems involving computer simulations using numerical methods and experimental determinations in soil columns being undertaken in this Department.

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3,360-Myr old gneisses from the South Indian Craton

R. D. Beckinsale

Institute of Geological Sciences, Gray's Inn Road, London WC1, UK

S. A. Drury & R. W. Holt

Department of Earth Sciences, Open University, Milton Keynes, UK

Reliably dated continental crust older than 3,000 Myr is only known from restricted segments of cratons in southern Africa, Greenland–Labrador, and Minnesota (summarised in ref. 1). These rare Archaean nucleii are very important in constraining the timing and mechanism of the differentiation of continental material from the mantle. We report here preliminary Rb–Sr whole rock data for Archaean gneisses from the Gorur-Hassan area, South Indian Craton which yield the oldest age yet reported from the Indian sub continent (3,358±66 Myr) with a very unradiogenic initial $^{87}Sr/^{86}Sr$ ratio (0.7000±0.0004) which implies that the igneous precursors of these gneisses were derived from a relatively Rb-depleted source within the mantle.

The gneisses of the Gorur–Hassan area (Fig. 1) are components of the polyphase Archaean Peninsular Gneisses of the South Indian Craton. They are unconformably overlain by the low grade volcanic-sedimentary association of the Dharwar supergroup, represented locally by the Bababudan and Shigegudda belts[2]. Their relationship to the supposed pre-Dharwar, high grade volcanic–sedimentary association of the Holenarsipur belt[2,3] is, as yet, ill defined. The areas to the west and east are characterised by marked late or post-Archaean shear belts in which gneisses and supracrustals are deformed together. To the north-west, the gneisses have undefined relations with a foliated granitoid mass, the Chickmagalur Granite, which is also unconformably overlain by the Dharwar supergroup of the Bababudan belt. Both the gneisses and the Holenarsipur supracrustals are intruded by a small post-kinematic granitoid mass the Mavinkere Granite, which may also post-date the major shear belts (Fig. 1).

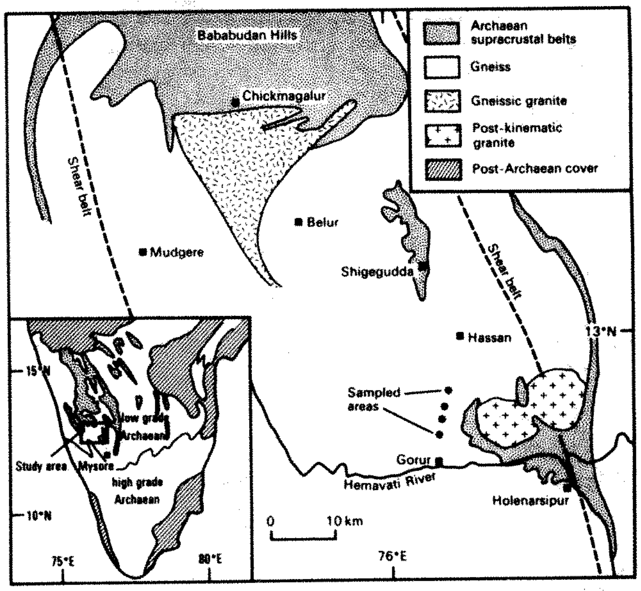

Fig. 1 Geological sketch map of parts of Hassan and Chickmagalur Districts, Karnataka.

The gneisses described here were chosen for reconnaissance dating because of their location in an area of low finite strain and the fact that they are stratigraphically the oldest known materials in the South Indian Craton readily amenable to the Rb–Sr method. The sample were all taken from active quarries and are extremely fresh rocks.

The gneisses range from dark dioritic amphibolites through tonalites and granodiorites to very pale trondjhemites. This gives the gneisses a broad banded aspect, each band showing little evidence of strong deformation. In places the compositional banding is emphasised by parallel highly strained zones with isoclinical folds. Late stage epidotic crush zones have developed locally. Analysed samples were collected from the least deformed representative of each phase, and at least 3 km from the post-kinematic Mavinkere Granite (Fig. 1).

The Rb–Sr data set out in Table 1 were determined at IGS by standard methods of mass and X-ray fluorescence spectrometry using an automatic V.G. Micromass 30 and an automatic Philips PW 1450. Data for standards obtained during the course of the sample analyses are also set out in Table 1. The Rb–Sr data are plotted on an isochron diagram in Fig. 2.

Least squares regression of the data points using the method of Williamson[4] yields a slope and intercept corresponding to an age and initial $^{87}Sr/^{86}Sr$ ratio of 3,369±31 Myr and 0.6995±0.0002 (1σ errors). The MSWD, indicating how well the points fit a line, using the 1σ errors estimated from replicate analyses (Table 1) is 15.2. Rejecting two samples, whose thin sections show slight epidotisation, reduces the MSWD to 1.84 and yields an age of 3,358±66 Myr (2σ) and an initial $^{87}Sr/^{86}Sr$ ratio of 0.7000±0.0004 (2σ) from the five-point isochron. Work is in

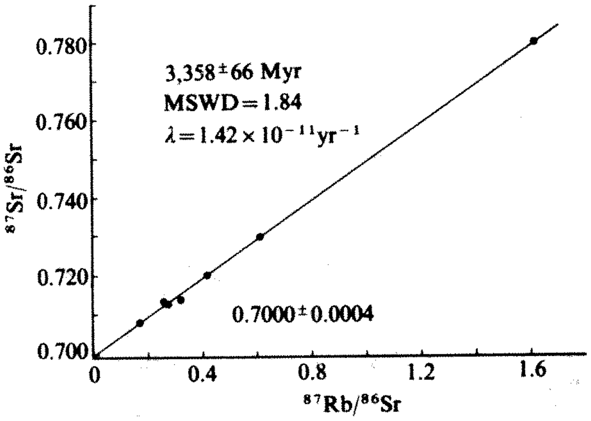

Fig. 2 Rb–Sr whole rock isochron plot for Gorur-Hassan gneisses. Errors are quoted at 2σ level.

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Table 1 Rb–Sr analytical data for Gorur-Hassan gneisses

Sample no. and rock type		Rb (p.p.m. ± 5%)	Sr	$^{87}Sr/^{86}Sr$ (±0.02%)*	$^{87}Rb/^{86}Sr$ (±1.0%)
I 291	grey-gneiss	56	966	0.70802	0.168
I 294	grey-gneiss	40	444	0.71300	0.259
I 285	biotite-trondjhemite	39	270	0.72008	0.415
I 290	pale-gneiss	42	201	0.72966	0.611
I 284	biotite-granodiorite	36	64	0.77946	1.624
I 293	grey-gneiss	54	565	0.71257	0.275†
I 288	biotite-amphibolite	19	176	0.71359	0.318†

Data for standards Eimer and Amend	$^{87}Sr/^{86}Sr$	Rb/Sr (weight)
$SrCO_3$ (19 runs)	0.70806 ± 1 (2 × s.e.)	
NBS 987 (14 runs)	0.71028 ± 2 (2 × s.e.)	
155725		0.691
GSP 1		1.100
G2		0.356
NBS 70A		8.053

* Normalised to $^{86}Sr/^{88}Sr = 0.1194$ and corrected to be relative to value of 0.70800 for the Eimer and Amend $SrCO_3$ by subtracting 0.00006.

† Not included in regression shown on Fig. 2.

progress to document further the Rb–Sr systematics of this crustal segment.

Previous whole rock Rb–Sr isochron ages for the Peninsular Gneisses range from 2,640 (ref. 5) to 3,250 Myr (ref. 6). The latter age was derived from three gneissic clasts in the basal Dharwar conglomerates and consequently has gross errors and little geological significance. However, it does suggest that gneisses of the age we report here may be widespread in the South Indian Craton.

The intial $^{87}Sr/^{86}Sr$ ratio of 0.7000 ± 0.0004 lies slightly below an $^{87}Sr/^{86}Sr$ growth line for the mantle (Rb/Sr = 0.03) and well below most similar Archaean gneisses from elsewhere (Fig. 3). Assuming that the initial ratio of the gneisses grew from a primordial ratio of 0.699 (ref. 7) at 4,560 Myr to their measured value at 3,358 Myr the Rb/Sr ratio during this time must have averaged about 0.020. This implies a source region for the igneous precursors of the gneisses depleted in Rb compared with more typical mantle (Rb/Sr = 0.038). Such a depletion may reflect either: (1) an earlier differentiation of material with high Rb/Sr ratios from the mantle, in other words it is possible that even older crustal rocks may be identified in the South Indian Craton; (2) a mantle source region that was originally anomalously low in initial $^{87}Sr/^{86}Sr$ in comparison with basaltic achondrites or other meteorites (ref. 7); (3) a source region that had been depleted in Rb/Sr by some indeterminate process. It is not possible to choose between these alternatives at present, although possibility (2) appears least probable.

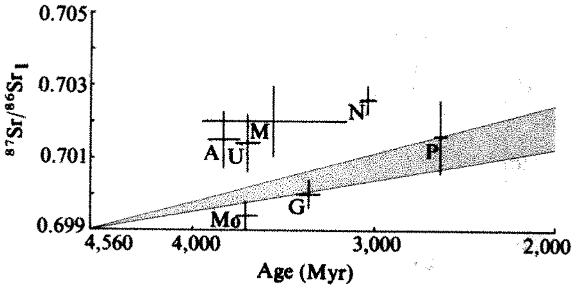

Fig. 3 Graph of initial $^{87}Sr/^{86}Sr$ versus age for Archaean gneisses relative to a range of $^{87}Sr/^{86}Sr$ ratios for igneous rocks derived from the mantle (stipple): A, Amîtsog gneiss, West Greenland[8]; N, Nûk gneiss, West Greenland[9]; M, Mashaba gneisses, Rhodesia[10]; U, Uivak gneisses, Labrador[11]; Mo, Morton gneiss, Minnesota[12]; P, Peninsular gneiss, Mysore[5]; G, Gorur–Hassan gneiss, this paper.

The very low initial $^{87}Sr/^{86}Sr$ ratio virtually rules out any evolution of the gneisses' Sr isotope system before the measured age. We therefore interpret the age of 3,358 ± 66 Myr to represent the age of separation of the gneisses' precursors from a mantle depth source region. The gneisses are the oldest rocks known from the Indian sub-continent. The anomalously low $^{87}Sr/^{86}Sr$ ratio of the gneisses and the reported presence of metapelitic and metabasic enclaves within the gneisses of the low finite strain zone of the Hassan District (B. Chadwick, personal communication) suggest the Hassan District to be potentially the most fruitful area for investigations of the early crustal history of the Indian sub-continent.

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Convergent metamorphism of eclogite and garnet-bearing ultramafic rocks at Lien, West Norway

L. G. Medaris Jr

Department of Geology and Geophysics,
University of Wisconsin, Madison, Wisconsin 53706

Eclogite and garnet peridotite and pyroxenite are important features in the supracrustal sequence of the Basal Gneiss region in West Norway. Three modes of origin have been proposed for these rock types: (1) both eclogite and garnet-bearing ultramafic rocks equilibrated in the upper mantle and subsequently were tectonically emplaced in their present positions[1–3], (2) the eclogites were produced by *in situ* metamorphism of a crustal sequence, but the ultramafic rocks originated in the upper mantle and were tectonically emplaced[4–10], and (3) the ultramafic rocks were derived from the mantle but were emplaced before the eclogite facies metamorphic event and have shared a common metamorphic history, at least in part, with the crustal eclogites[11]. I report here the application of recently developed geothermometers/barometers to garnet-bearing assemblages in the Lien peridotite at Almklovdalen and to adjacent eclogites in surrounding supracrustal gneisses which has documented a retrograde metamorphic trend from 820 °C, 28.1 kbar to 645 °C, 17.6 kbar in the ultramafic rocks and a prograde trend in the eclogites which converges with that of the ultramafic rocks. The Lien peridotite seems to have been derived from the upper mantle in eclogite facies conditions and emplaced into unusually thick continental crust during an eclogite facies metamorphic event.

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Table 1 Ranges in chemical composition of eclogite facies minerals in garnet peridotite, garnet pyroxenite, and eclogite

	Peridotite		Pyroxenite		Eclogite	
Olivine						
Fo	87.8–91.9		88.4–91.0			
Orthopyroxene	Cores	Rims	Cores	Rims	Cores	Rims
Wo	0.3–0.5	0.3–0.3	0.3-0.3	0.3–0.2		
Fs	10.8–7.7	10.9–8.1	12.7–8.0	12.6–9.4		
En	88.9–91.8	88.9–91.6	87.0–91.7	87.2–90.4		
Al_2O_3(wt %)	0.98–0.59	0.71–0.55	1.02–0.46	0.65–0.45		
Clinopyroxene						
$NaCrSi_2O6$	4.9–3.2	3.9–3.1	1.1–3.4	1.1–0.9	0.3–0.2	0.1–0.0
$NaFe^{3+}Si_2O_6$	1.3–1.8	1.5–1.8	2.1–1.2	1.8–1.6	4.5–2.3	4.4–4.6
$NaAlSi_2O_6$	8.8–3.6	3.3–2.1	11.2–8.3	9.4–6.1	36.0–33.9	37.6–29.8
$CaTiSi_2O_6$	0.1–0.2	0.1–0.1	0.2–0.1	0.2–0.0	0.2–0.3	0.4–0.4
$CaAl_2SiO_6$	1.3–1.9	2.6–2.0	0.4–0.9	0.0–1.0	0.1–0.8	3.1–3.9
$CaMgSi_2O_6$	76.3–81.3	80.7–83.7	76.0–80.3	78.6–84.0	52.1–53.8	47.5–54.7
$CaFeSi_2O_6$	3.0–4.1	5.1–4.5	5.1–2.9	5.0–3.9	5.2–7.4	5.5–5.4
$Mg_2Si_2O_6$	4.2–3.7	2.7–2.6	3.6–2.8	3.7–2.4	1.4–1.2	1.2–1.1
$Fe_2Si_2O_6$	0.1–0.2	0.1–0.1	0.2–0.1	0.2–0.1	0.1–0.1	0.2–0.1
Garnet						
Py	61.9–69.3	59.9–69.2	59.2–71.9	58.6–70.1	21.1–35.1	29.0–40.8
Alm + Sp	22.5–18.1	27.0–18.3	29.2–16.1	29.7–17.9	55.0–45.0	43.1–39.3
Gr	12.6–12.6	13.1–12.5	11.6–12.0	11.7–12.0	23.8–19.9	27.9–19.9

One of the classic occurrences of garnet peridotite and pyroxenite in west Norway is located within the Lien peridotite[1-2,12-13] (Fig. 1), which is part of a large ultramafic body in Almklovdalen[14]. The Lien peridotite consists predominantly of partly serpentinised, foliated chlorite peridotite containing an equilibrium assemblage of forsterite–enstatite–tremolite–clinochlore ± low Al chromite with a fine- to medium-grained mosaic texture. Four areas of garnet peridotite with locally abundant garnet pyroxenite layers are preserved within the Lien body (Fig. 1) and represent relicts of the earliest discernible mineral assemblage in the ultramafic mass. Garnet peridotite displays an inequigranular texture in which large strained grains of clinopyroxene, orthopyroxene, and garnet occur in a fine- to medium-grained mosaic matrix of strain-free olivine, orthopyroxene, clinopyroxene, pargasitic amphibole, and small amounts of chromiferous spinel. Garnet pyroxenite displays similar texture and mineralogy, except that olivine is subordinate in amount, or absent. In both rock types garnet is commonly surrounded by a kelyphitic rim of finely intergrown amphibole and spinel.

The Lien peridotite has generally concordant contacts with surrounding gneisses of tonalitic to granodioritic composition. Garnet- and muscovite-bearing layers are present in the gneiss, and amphibolite layers and boudins are common. Amphibolite facies mineral assemblages in the gneiss are compatible with the chlorite peridotite assemblage in the ultramafic rocks, which is stable from 700 °C to 780 °C (ref. 15).

The predominant structural style in gneiss and chlorite peridotite is one of tight to isoclinal similar folds with a penetrative axial plane foliation which strikes east-northeast and dips steeply to the south. Linear elements of these folds measured in both rock types over the map area plot along a girdle which coincides with orientation of the axial plane foliation in the area. Thus, not only do gneiss and chlorite peridotite contain compatible mineral assemblages, they also share the same structural elements.

Nine occurrences of eclogite boudins, the largest of which is 20 m by 50 m, have been located in gneiss adjacent to the Lien peridotite (Fig. 1). Eclogite contains a primary mineral assemblage of garnet–omphacite–edenitic amphibole ± zoisite ± biotite–quartz–rutile–apatite–sulphide ± carbonate. Retrograde recrystallisation has been extensive in some samples, largely due to development of amphibole–plagioclase symplectite from omphacite and amphibole kelyphite from garnet.

Electron microprobe analyses were obtained for cores and rims of minerals in five samples of garnet peridotite, seven of

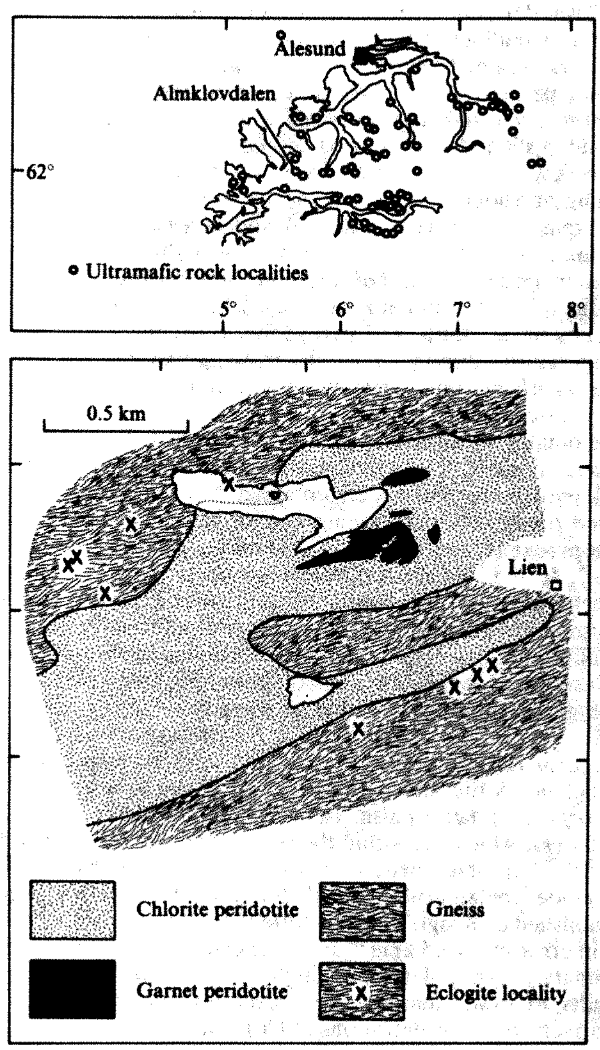

Fig. 1 Locality and geologic map of the Lien peridotite in Almklovdalen, west Norway.

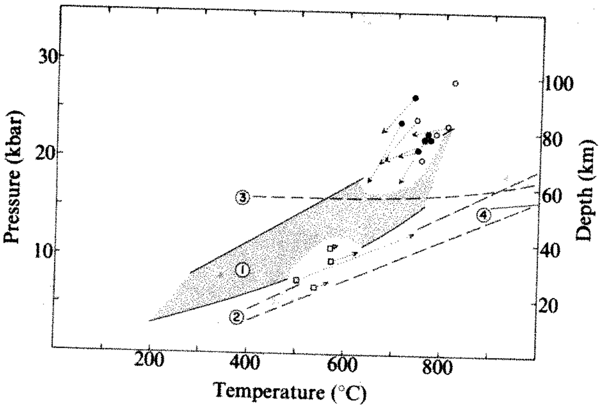

Fig. 2 Estimates of pressures and temperatures for garnet peridotite, garnet pyroxenite, and eclogite at Lien. Stippled area 1, prograde metamorphic trend for eclogites in Basal Gneiss of west Norway[8]; stippled area 2, field for transition of basic granulite to eclogite[4]; 3, boundary separating stability fields of garnet lherzolite and spinel lherzolite[20]; 4, garnet lherzolite-spinel lherzolite transition in the system CaO-MgO-Al_2O_3-SiO_2 (ref. 21) ○, Core compositions of minerals in garnet peridotite; ●, cores in garnet pyroxenite; □, cores in eclogite; arrows, rim compositions of minerals.

garnet pyroxenite, and four of eclogite (summarised in Table 1). Fe^{3+} was determined by charge balance calculations and by wet chemical analyses of selected mineral separates.

Coarse-grained garnet, clinopyroxene, and orthopyroxene in garnet peridotite and pyroxenite are compositionally zoned; from core to rim there is an increase in Fe and decrease in Mg in garnet, a slight decrease in Al and Cr in orthopyroxene, and a decrease in Al, Cr, and Na in clinopyroxene. Compositional zoning in eclogite garnet is opposite to that in peridotite and pyroxenite, that is, a decrease in Fe and increase in Mg from core to rim. Zoning in eclogite clinopyroxene is slight, there being a small increase in Al and decrease in Ca from core to rim.

Due to compositional zoning $K_{\mathrm{D\,gar/cpx}}^{\mathrm{Fe/Mg}}$ increases from cores to rims in peridotite and pyroxenite, reflecting a decrease in temperature during mineral growth, whereas $K_{\mathrm{D\,gar/cpx}}^{\mathrm{Fe/Mg}}$ decreases from cores to rims in eclogite, indicating an increase in temperature.

Estimates of equilibration temperatures for core and rim compositions of coarse-grained minerals in garnet, peridotite and pyroxenite have been obtained from geothermometers based on Fe-Mg partitioning among garnet, orthopyroxene, clinopyroxene, and olivine[16,17] and on the pyroxene miscibility gap[18]. In Fig. 2 temperatures based on $K_{\mathrm{D\,gar/cpx}}^{\mathrm{Fe/Mg}}$ have been selected because this is the most sensitive of the various geothermometers in the temperature range of interest. Pressures of equilibration have been calculated according to Wood's[19] procedure, based on the Al content of orthopyroxene in equilibrium with garnet and incorporating all corrections for Cr and Fe^{3+}. Calculated values of pressure and temperature are consistently lower for rim compositions compared to core compositions in garnet peridotite and pyroxenite and reveal a clear trend of retrograde metamorphism from 820 °C, 28.1 kbar to 645 °C, 17.6 kbar, which lies within the stability field of garnet peridotite[20,21], but which penetrates the higher *P–T* portion of the prograde metamorphic trend for eclogites in this region established by Krogh[8] (Fig. 2). Although pressure estimates may be in error up to ±5 kbar[18] due to uncertainties in temperature estimates, extrapolation of high temperature experimental results to lower temperature conditions, and the effects of relatively high concentrations of Cr in garnet and pyroxenes, it seems most likely that the Lien garnet peridotite and pyroxenite equilibrated in response to decreasing pressures of the order of 25–20 kbar and certainly <30 kbar. The *P–T* estimates obtained in this study are in close agreement with those reported recently by Carswell and Gibb[11] for Norwegian crustal garnet lherzolites.

Neither orthopyroxene, phengite, nor primary plagioclase occur in eclogites close to the Lien peridotite, and consequently, only minimum estimates of equilibration temperatures and pressures can be made[22]. Core and rim compositions of adjacent garnet and omphacite grains in four eclogite samples define a prograde metamorphic trend (Fig. 2), based on experimental calibration of Fe-Mg partitioning between garnet and clinopyroxene in basic rocks[22,23] and the jadeite content of omphacite[24]. Considering that only minimum pressure estimates can be made for these samples, their metamorphic pattern is consistent with that for other eclogites in the region. Furthermore, values for $K_{\mathrm{D\,gar/cpx}}^{\mathrm{Fe/Mg}}$ in the four eclogite samples (5.2, 8.0, 9.9, and 10.7) are compatible with values (5.5–9.5) which would be expected in basic rocks recrystallised in the same *P–T* conditions as those for the Lien garnet peridotite and pyroxenite.

This investigation supports a dual origin for eclogite and garnet-bearing ultramafic rocks at Lien, eclogite having developed by *in situ* metamorphism of crustal materials and ultramafic rocks having been derived from the upper mantle. Such an interpretation was proposed previously by Bryhni *et al.*[10] and Carswell[9] and subsequently supported by Brueckner's[25] strontium isotope analyses of clinopyroxene from eclogite and garnet-bearing ultramafic rocks. The metamorphic trends for eclogite and ultramafic rocks suggest that eclogite facies metamorphism of the ultramafic rocks may have begun in the upper mantle and then continued at progressively decreasing temperatures and pressures as the ultramafic rocks were emplaced in continental crust at depths of 60–70 km and that eclogite and garnet peridotite and pyroxenite have shared in the same metamorphic episode.

This investigation and published isotopic data indicate the following three-stage evolution of the Lien peridotite: (1) emplacement of mantle-derived garnet-bearing ultramafic rocks into a deep crustal environment and convergent metamorphism with eclogite 1,850 Myr ago[26]; (2) extensive recrystallisation of the Lien peridotite and surrounding gneisses in amphibolite facies conditions 1,150 Myr ago[27]; and (3) isotopic re-equilibration of the gneisses and development of hydrous fracture and contact mineral assemblages in peridotite during the Caledonian Orogeny[27,28].

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Size constancy fails below half a degree

John Ross*, B. Jenkins* & J. R. Johnstone†

Department of Psychology* and Department of Physiology†, University of Western Australia, Nedlands, Western Australia, 6009, Australia

Size constancy is a term used to refer to the fact that objects seem to maintain size as their distance from an observer changes, despite change in image size. it is known that size constancy has limits[1,2]. People seen from the top of tall buildings look small. By observing objects, including the Moon, and after-images, we demonstrate here that size constancy holds when image size is above half a degree, and breaks down when it is less. In other words, image size is scaled for distance only when image size is above half a degree.

The primary evidence for size constancy is negative: objects do not seem to change in size with change in their image size. An immediate experimental difficulty is that observers can judge both object size and image size separately. An observer who is asked if an object seems to change size as it moves away from him may reply 'yes', meaning, not that the object changes in apparent size, but that it covers a smaller patch of the visual field. For this reason, measurements obtained from naive subjects are particularly suspect in the field of size constancy. It is safer, therefore, to rely on observations that the reader can make and repeat himself, rather than what was said by experimental subjects, who may well have been confused about what was asked of them.

It may readily be observed, without the aid of instruments, that large objects begin to look small when their image size reduces to half a degree, or less. A convenient reference is the fingernail of the index finger, which subtends about a degree with the arm fully extended. People seen in the distance seem doll-like[2] when their image size is less than half the size of the image of the fingernail. Aeroplanes, after taking off, maintain object size and then, at a certain point, look small. The change occurs when image size shrinks to half a degree. Similarly, motor cars, lorries, trains and houses, seen from the air, look small, like toys, when their images subtend less than half a degree. As a plane comes in to land a passenger may observe the sudden transition from small to large occurring for each object as its image size breaks the half a degree barrier. Many other examples will be noticed by the reader as soon as he is alerted to half a degree as the critical image size, and learns to measure image size with his fingernail. The situation is less clear with smaller objects, although to the authors, household and laboratory objects look to have diminished in size at a distance at which each first subtends less than half a degree.

The proposition that size scaling holds only when image size is above half a degree may be tested by changing apparent distance while holding image size constant. One convenient method of so doing is to form an after-image, and observe it as projected on to surfaces at different distances. Emmert's law[3] states that the apparent size of an after-image varies directly with the perceived distance of the surface on which it is projected. We have observed that Emmert's law breaks down when after-image size is less than half a degree. An after-image does not seem to change size with the distance of the surface on which it is projected when it is below the critical size of half a degree. The reader is urged to verify this observation, which he can do conveniently by observing the after-image formed by a flashgun, from a distance at which it subtends half a degree or less.

An alternative way of demonstrating this to a large group of people, which we have used several times, is as follows. A flashgun is so positioned that it subtends more than half a degree to roughly half the group, and less to the other half. All are asked to observe the after-image on the palm of the hand and then on a side wall of the room. They are then asked to raise their hands if the image seemed to change in size when transferred from the near to the far surface. With high probability, subjects w images above half a degree (those in the front of the room) raise their hands and subjects with images below half a degree (those in the back of the room) do not, despite the fact that they can see raised hands in front of them. Results are well beyond chance levels, so much so that the experiment may be used as a classroom demonstration of Emmert's law and of its boundary condition.

The Moon is of interest in this connection, because it subtends almost exactly half a degree, whatever its position in the sky, and because it seems large at the horizon and small when high in the sky. Variation in apparent distance is the most probable cause[4] of the Moon's variation in apparent size. According to the hypothesis that size scaling occurs at half a degree and above, but not below, the illusion of change in the Moon's size should be abolished by reducing the size of image. This can be done optically. The full Moon was observed in a clear sky at Easter, 1978 (and subsequently) through a positive lens close to the eye, and a negative lens further away (reversed galilean telescope), both contained in a tube and so positioned that the Moon's image was reduced by about one-third, to 20 arc min. The horizon Moon seemed to diminish by much more than a third in diameter, by a factor of two or three. The zenith Moon, when it was observed, seemed to diminish by only the expected one-third, like terrestrial objects whose distance can be judged by eye. Furthermore, the zenith Moon, when reduced, seemed to be of the same size as the horizon Moon, when it was reduced. These effects are not due to observing through a tube. The horizon Moon seemed much larger than the zenith Moon when both were observed through a tube containing no lenses. It may be relevant that, of all Jupiter's moons, only one, Io, subtends a visual angle near half a degree at Jupiter's surface. Thus, only Io should seem to change size to an observer on Jupiter.

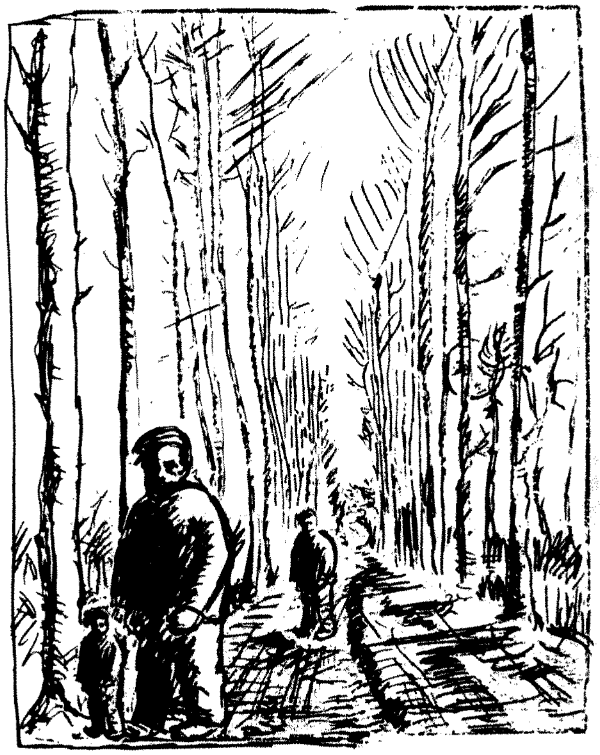

Fig. 1 Because the figure in the background seems more distant than the small figure in the left foreground, its size seems larger when the page is at normal reading distance. At this distance both figures subtend more than 30 arc min in the vertical. At a greater distance, when both subtend less than 30 arc min, the two seem to be the same size. The head of the large figure also looks smaller than the Moon, which can be seen on the right of the picture. The head and Moon also seem the same size when they subtend 30 arc min or less. As the observer moves back from the picture, this critical image size is reached earlier for the Moon and the head than it is for the two smaller figures.

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xample of the fact that image size is critical is Fig. 1. Three men are depicted (after Van Gogh), the same size. Perspective makes the one in the ground seem further away, and larger at normal reading distance. However, when the page is removed to a distance (about 3 m) at which both figures subtend 20 arc min, they seem the same size.

There may be some significance in the fact that size constancy breaks down at half a degree. It has been proposed[5,6] that there are no size tuned channels in the low frequency range, but instead a single mechanism sensitive to luminance gradients: above the peak of the contrast sensitivity function there is harmonic analysis and below it contrast gradient analysis. The hypothesis may therefore be advanced that large and small retinal images are calibrated in different ways, the large being subject to automatic size correction to take account of distance, and the small giving absolute size information, which is not corrected for distance.

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A dorso-ventral shift of embryonic primordia in a new maternal-effect mutant of *Drosophila*

Christiane Nüsslein-Volhard

European Molecular Biology Laboratory, 69 Heidelberg, FRG

Margit Lohs-Schardin & Klaus Sander

Institut für Biologie I (Zoologie), Universität Freiburg

Christoph Cremer

Institut für Humangenetik, Universität Freiburg, 78 Freiburg, FRG

Mutations altering the spatial pattern of embryonic primordia are valuable tools for studying the earliest steps of embryonic pattern formation. As the spatial organisation of the egg cell arises during oogenesis, such mutants are likely to affect the embryo via the maternal genome (maternal effect). In *Drosophila*, most maternal-effect mutants studied so far lead to early developmental arrest. A few others yield pattern defects apparently linked to local failure of blastodermal cellularisation rather than to abnormal arrangement of primordia or ooplasmic determinants[1,2]. A small number of mutants, however, are known in which the embryonic fate map is affected, for example, *bicaudal*[3,4], which profoundly alters the antero-posterior pattern of embryonic organisation. The *bicaudal* phenotypes suggest that the mutation causes a perturbation of a morphogen gradient that defines the antero-posterior coordinate in early embryonic pattern formation[5,6]. A new maternal-effect mutant, *dorsal*, seems to exert a similar influence on the dorso-ventral coordinate for patterning[6]. *Dorsal* (*dl*) has both a recessive and a dominant maternal-effect phenotype. The *dl* recessive phenotype is marked by an apparently complete lack of dorso-ventral polarity from early embryogenesis onward[6]. The *dl* dominant (dl^{D}) phenotype, which we report on here, is temperature sensitive: at 22 °C, *dl*/+ females lay eggs which develop normally while eggs produced at high temperature (29 °C) yield abnormal larvae which fail to hatch. These mutant dl^{D} larvae vary in phenotype but nearly always lack muscles and frequently part of the ventral hypoderm. They are defective in structures that arise from the ventral region of the blastoderm in normal embryogenesis[6,7] (Fig. 1).

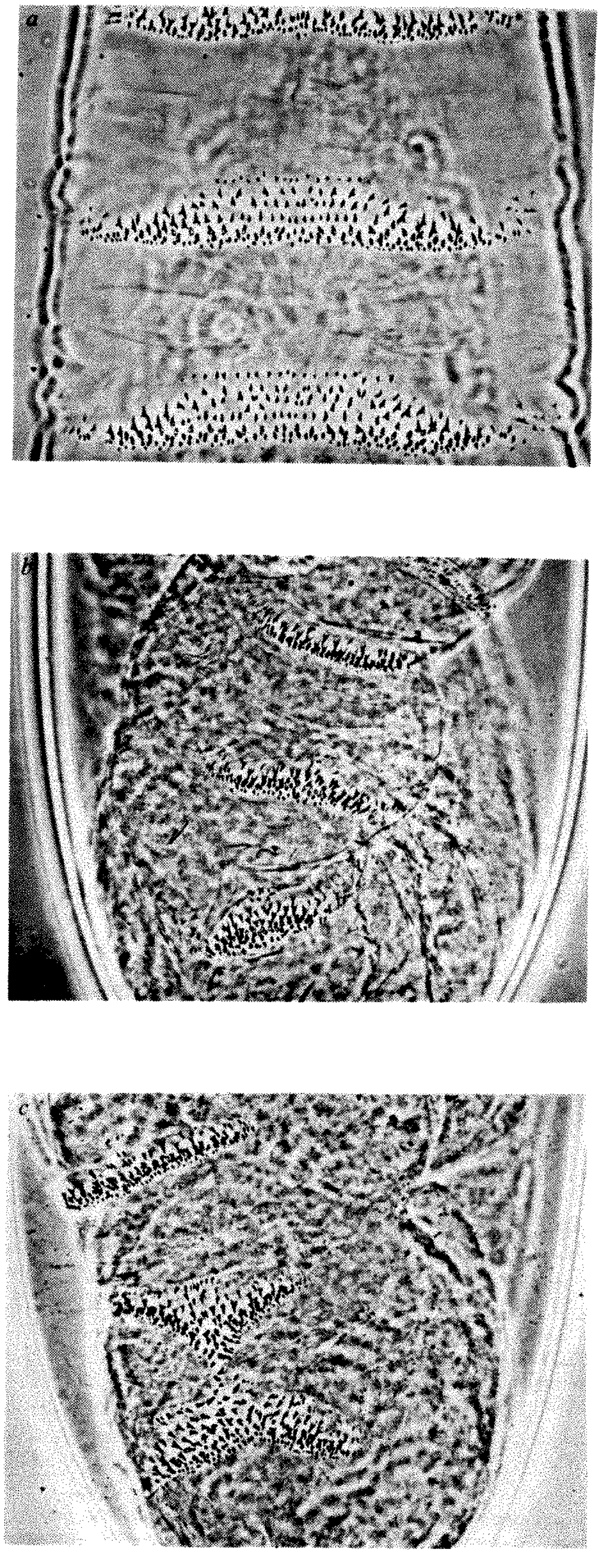

Fig. 1 Ventral hypoderm from the abdomen of wild-type and mutant larvae. *a*, Larva shortly after hatching; *b*, *c*, dl^{D} larvae produced by *dl*/+females at 29 °C. The genotype of the females was *al dp b dl bic sp*/*vg*$^{\mathrm{D}}$ in all experiments. For the descriptions of genes see refs 6, 10. The expressivity of the dl^{D}-phenotype is dependent on the genetic background of the female and this particular genotype was chosen because it happened to produce weak and extreme phenotypes with approximately equal frequency, while less than 1% of the eggs hatch. Larvae were regarded as extreme mutant if they had a notably reduced width of the ventral denticle hook rows (*b*); they also always were twisted or coiled up in the egg case. The larva in *c* has been irradiated mid-ventrally at 40% egg length (0% = posterior pole) at the cellular blastoderm stage.

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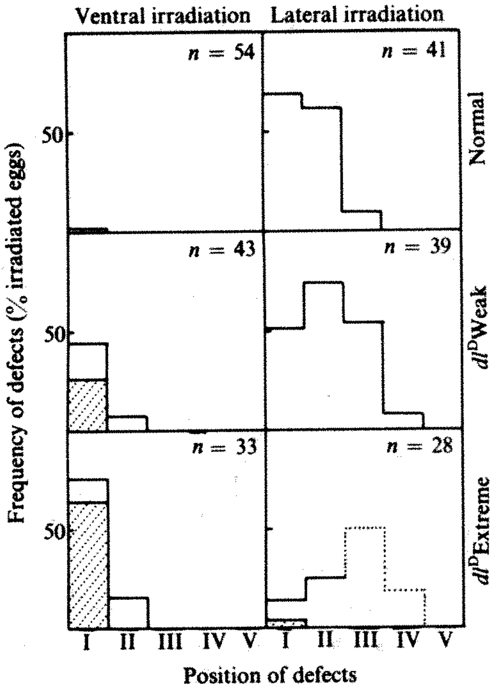

Fig. 2 Distribution of defects in the ventro-dorsal hypoderm pattern following UV-laser irradiation of wild-type and mutant eggs at the blastoderm stage. Eggs were collected from *dl*/+ females (*al dp b dl bic sp*/*vg*$^{\mathrm{D}}$) or +/+ (Oregon K) females at 29 °C or 25 °C respectively in 30-min intervals, aged until briefly before blastoderm formation, partly dechorionated and irradiated with a UV-laser microbeam (257 nm, 20 μm diameters, 7 erg) at the cellular blastoderm stage as described[9]. Ventral irradiations (left panels) were aimed at the ventral midline of eggs orientated with their ventral side facing the UV beam. For lateral irradiations, laterally orientated eggs were irradiated at 30% egg diameter measured from the ventral side (vd4 in ref. 9). These positions correspond to 0% or 36% half-circumference (0% = ventral midline, 100% = dorsal midline). All eggs were irradiated at 30% egg length (0% = posterior pole). This position was chosen because it creates defects predominantly in the posterior abdomen (centre of defect 5th abdominal segment), a region permitting optimal detection of defects also in extreme mutant larvae which often are twisted or coiled in the anterior abdominal and thoracic region. Irradiation at more anterior position (40% and 50% egg length) gave similar results (data not shown). Following irradiation, more than 90% of the eggs survived to the larval stage. The larvae and unhatched eggs were fixed and cleared and the cuticle examined for defects using phase contrast optics at ×400 magnification (Zeiss Neofluar). For quantitative evaluation of the location of the defects the ventro-dorsal cuticular pattern was divided into five sections[9] (I and II, ventral and lateral half of the ventral hypoderm; III, lateral hypoderm; IV and V, lateral and dorsal half of dorsal hypoderm, see ref. 9 for description of markers used). The frequency of defects per section was determined whereby defects spanning more than one section were counted separately for each section. Mutant larvae were regarded as extreme if the width of the ventral denticle belts was markedly reduced. Owing to a severe twisting of the hypoderm in extreme mutant larvae, the lateral and dorsal hypodermal region (sections III to IV) could sometimes not reliably be scored, the values indicated by stippled bars are therefore lower limits of the true defect frequency. The shaded bars indicate the frequency of bilateral defects, in which both right and left sides of the ventral hypoderm were affected simultaneously. No correction was made for spontaneous defects (<5% in both mutant and wild-type larvae), however, larvae with defects outside the abdominal segments A3–A7 (<5%) were disregarded in the calculations.

The absence of ventrally derived structures in dl^{D} embryos is not due to a failure of cell formation in ventral egg regions. The embryos develop an apparently normal cellular blastoderm. At gastrulation, the first deviation from normal development is observed: dl^{D} embryos fail to form the ventral invaginations which lead to the separation of the mesodermal and anterior endodermal anlagen from the ectoderm in normal development[6]. The defect of ventral structures could thus result from inability of the ventral cells to develop beyond blastoderm. Alternatively, the ventral cells could give rise to structures normally derived from more lateral egg regions, which would mean that the dorso-ventral fate map is affected by the mutation. To test this idea, we investigated the developmental fate of blastoderm cells in dl^{D} embryos. As a method for fate mapping, we used UV-laser induced local lesions.

We have previously shown that irradiating wild-type blastoderms with a UV-laser microbeam[8] frequently leads to localised cuticle defects in the ensuing larvae[9]. The position of the defects within the cuticle pattern depends rather strictly on the site of irradiation, indicating that the defects were created by damaging the respective anlage cells. We have used this correlation to construct a detailed fate map of the larval epidermis[9] (Fig. 3). The mid-ventral region (20% of the egg circumference) gives rise to mesoderm[7] and irradiation of this region very rarely induces cuticular defects. The larval hypoderm stems from the larger part of the blastoderm (76%); ventro-lateral and lateral irradiations create defects predominantly in the ventral hypoderm, while the dorsal hypoderm is frequently defective following dorso-lateral and dorsal irradiations.

When dl^{D} embryos are irradiated as blastoderms, the pattern of the ensuing defects is markedly changed (Fig. 2). Following mid-ventral irradiations, 79% (26/33) of the extreme mutant larvae show a defect in their ventral hypoderm while only 2% (1/54) of the control larvae do so. Larvae of a weak mutant phenotype give an intermediate value (20/43). Moreover, the majority of defects are bilateral defects, impairing right and left side of the ventral hypoderm simultaneously, while ventral hypodermal defects created in normal larvae are always restricted to one side, consistent with the separation of the right and left ventral anlagen by the mesodermal anlage. This result

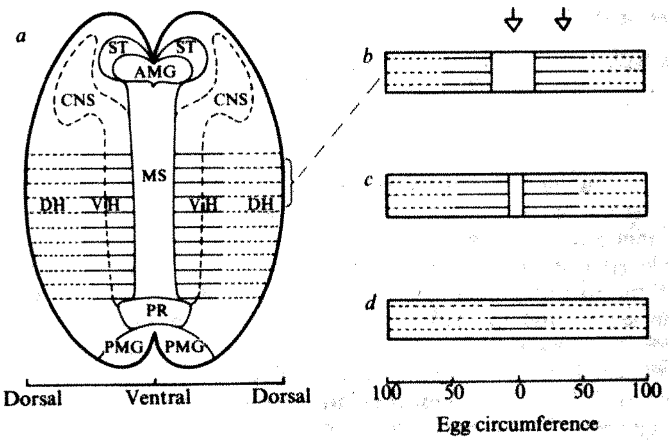

Fig. 3 Alteration of the embryonic fate map in *dl*-dominant embryos. *a*, Poulson's fate map[7] of the *Drosophila* egg cut open along the dorsal midline and spread out. Simplified and slightly modified[9,11]. The location of the segment borders in the larval hypoderm as determined from laser-induced lesions[9] are indicated by the transverse lines, dotted for the dorsal part of the segments. The serosal anlage is not indicated separately because it seems that at least part of it is later incorporated in the dorsal hypoderm. AMG, anterior midgut; PMG, posterior midgut; ST, stomodeum; CNS central nervous system; PR, proctodeum; MS, mesoderm; VH, ventral hypoderm; DH, dorsal hypoderm. *b–d*, Proposed alteration of the fate map in *dl*-dominant embryos; *b*, middle region of fate map in a normal embryo; *c*, in a dl^{D} embryo with weak; and *d*, with extreme mutant phenotype. The left and right anlage for the ventral hypoderm, flanking the mid-ventral mesodermal anlage, are shifted ventrally at the expense of the mesoderm anlage. The absence of anterior midgut and malpighian tubules (part of the posterior midgut[7]), in extreme mutant embryos suggests that the shift of lateral primordia towards the ventral midline also occurs at the anterior and posterior egg regions. The arrows indicate the sites of irradiation with respect to the egg circumference.

ventral cells which form mesoderm in normal instead form ventral hypoderm in dl^D embryos. iations indicate that not only the fate of ventral, lateral cells is affected in mutant embryos. Figure 2 shows that in contrast to the high defect frequency in control embryos (88%) relatively few dl^D embryos with extreme phenotype have defects in their ventral hypoderm (7/28), most of the defects being located in the dorso-lateral part of the hypoderm. This shift of defect location towards more dorsal positions in the hypodermal pattern is less pronounced in weak mutant phenotypes. Note that in both ventral and lateral irradiations, the antero-posterior positions of the defects are very similar in mutant and wild-type embryos and correspond to the site of irradiation.

The results suggest that the absence of ventral structures in dl^D embryos results from a change of the embryonic fate map: ventral cells form ventral hypoderm instead of mesoderm, while lateral cells form hypodermal structures that are normally derived from more dorsal positions. It seems that the lateral and dorsal anlagen are shifted towards the ventral midline at the expense of ventral anlagen. The degree of this shift parallels expressivity of the mutant phenotype. In extreme cases, it may be as much as 30% of the dorso-ventral coordinate as judged from the width of the remaining ventral hypoderm (Fig. 1) and by comparing the distribution of defects following lateral irradiations of extreme mutant larvae to that obtained in normal larvae by more dorsal irradiations[9]. The proposed alteration of the fate map is shown in Fig. 3.

We suggest that *dorsal*, rather than eliminating a localised determinant required for proper ventral development, affects a continuous property, such as a morphogenetic gradient, that defines the entire dorso-ventral coordinate of the developing embryo.

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Intrinsic immunosuppressive activity of different trypanosome strains varies with parasite virulence

David L. Sacks*, Murray Selkirk, Bridget M. Ogilvie & Brigitte A. Askonas*

Divisions of Immunology* and Parasitology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7, UK

The virulence of African trypanosomes varies greatly with the host and parasite strain combinations used[1]. In mice, infections range from those which are rapidly fatal to those which more closely resemble the chronic disease patterns seen in man and domestic cattle[2]. The basis of trypanosome virulence is assumed to be related to intrinsic parasite replication rate and the effectiveness of the host's immune response to successive waves of parasites which differ in their surface antigens. Continued control of each emergent population requires sustained immune vigilance. Thus, the generalised immunodepression which accompanies African trypanosomiasis in man[3], cattle[4] and experimental animals[5,6] would be expected to determine the fatal outcome of most untreated infections if the secondary immunodeficiency extends to responses against the parasite as well as to unrelated antigens. Virulence, in this view, could be explained by the rapidity and intensity with which a given strain of trypanosomes induces immunodepression in a given host. We have attempted to define the relationship between parasite virulence and generalised immunodepression by examining the intrinsic potential of trypanosome strains of differing virulence to induce immunodepression in the same murine host. Following the observation that kiled trypanosomes or their subcellular membrane fractions mimic the immunological consequences of active infection *in vivo*[7], it has now become possible to compare the immunosuppressive potential of acute and chronic strains by using active fractions derived from identical numbers of killed organisms. We conclude from these comparisons that trypanosome strains can have inherently different immunosuppressive activities, and that the extent to which IgM responses, in particular, are suppressed correlates with and suggests a basis for parasite virulence.

The parasitaemia profiles of four clones from three strains of *Trypanosoma brucei brucei* (see Fig. 1 legend) ranging in virulence from acute (NIM 9) to subacute (NIM 2 and 6) and chronic (NIM 7), are presented in Fig. 1. The association of fluctuating parasitaemias with the emergence of antigenically distinct parasites has already been described for clones NIM 2 and 6 (ref. 2) and for the chronic strain from which clone NIM 7 is derived[8]. Taken as a whole, the greater the level of initial parasitaemia, the shorter the survival time of the infected mice. In addition, the prepatent period, that is, the time from inoculation until first detectable blood parasitaemia, was shortest for clone NIM 9, for which even the initial parasitaemia was uncontrolled. The subacute and chronic clones each had indistinguishable prepatent periods of between 3 and 4 d, suggesting that their intrinsic replication rates were similar. This was confirmed by infection of irradiated mice in which no significant difference was observed between growth rates of NIM 2, 6 or 7 parasites in either blood or tissues (data not shown). Growth of clone NIM 9 was, as expected, significantly more rapid than that of the other clones in irradiated animals.

Membrane fractions derived from carefully determined numbers of organisms of each clone were prepared and injected into mice 4 d before priming with sheep red blood cells (SRBC). A large proportion of trypanosome material is routinely lost during the fractionation procedures. However, the final membrane yield was comparable between each of the four clones; reproducibly, 20–30 μg protein was derived from 10^7 trypanosomes. The comparative suppressive effect of each fraction on the direct and indirect anti-SRBC plaque-forming cell (PFC) response is shown in Fig. 2. IgG response, measured by indirect PFC, seemed to be extremely sensitive to the effects of all four clones. Membrane fractions derived from 10^8 parasites of each of the four clones inhibited up to 98% of the normal 1gG response. Fractions from 10^7 parasites were slightly less immunosuppressive, but the degree of suppression was still the same for all four clones. However, at this same dose, we were able to detect a strict hierarchy with respect to inhibition of IgM responses measured by direct PFC. The pattern of IgM suppression induced by the membrane fractions was directly correlated with the virulence of the trypanosome strains from which they had been derived. At a log higher dose, the acute and subacute clones each had potent suppressive effects (70–80%), whereas the inhibitory activity of the chronic strain remained significantly lower (35%).

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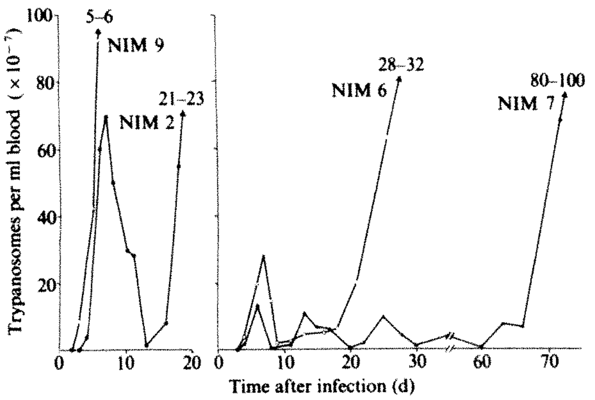

Fig. 1 Course of infection with four clones of *T.b. brucei* in (CBA/H$\times$C57BL/6)F_1 female mice, and survival times. Each point represents the mean parasitaemia of five or six mice, except for NIM 7, which represents the parasitaemia of a representative individual mouse. Trypanosome passage and cloning, and infection of mice with 200 parasites, were as described previously[12]. Clones NIM 2 and NIM 6 are non-cross-reacting variant clones, both derived from primary isolate Serengeti/66/SVRP/42 (ref. 2), previously referred to as strain S42. Clone NIM 7 is derived from primary isolate Uganda/66/EVE/10, and NIM 9 is derived from primary isolate Uganda/60/EATRO/217. The nomenclature follows that recently proposed for the reference of salivarian trypanosomes[13]. Parasitaemias were followed by examination of wet tail blood films under high power and counts converted to trypanosomes per ml as previously described[12].

The comparative suppression induced during active infection is summarised in Table 1. When mice were infected with 200 parasites of clones NIM 2, NIM 6 or NIM 7 and challenged with SRBC 10 d later, the IgG antibody response was completely inhibited regardless of the relative virulence of the infecting clone. The suppression of IgM antibody was virtually complete (background levels) for those clones which led to uncontrolled parasitaemia soon after day 10. Only the IgM response of the chronically infected mice, which continued to control parasitaemia for up to 80 d, survived at least partially intact (17% of normal response).

Although these data are concerned with the effects of trypanosomes on the response to an unrelated antigen, a similar pattern of suppression of the response to the parasite itself is suggested by the findings that the intrinsic differences between *T. brucei* clones in suppressing primary immune responses, particularly IgM responses, correlate well with trypanosome virulence, and the extent of IgM suppression induced during infection correlates with the control of parasitaemia and the pattern of infection in that host.

Whereas IgG responses are readily and comparably eliminated by active fractions of all four clones regardless of virulence, IgM responses are less susceptible to inhibition, and the degree of suppression varies directly with parasite virulence. In infected mice, our results and those of Hudson and Terry[8] essentially reflect these suppression patterns; thus, heterologous IgG responses are rapidly eliminated during both acute and chronic infections, whereas residual IgM responses persist during chronic infections in association with continued control of parasitaemia. These findings suggest that control of circulating trypanosomes can be achieved in the absence of IgG antibody by low levels of IgM. There is direct evidence to support this view. The control of blood parasites by nude mice occurs without detectable levels of IgG antibody[9], and very low concentrations of IgM antibody have been shown to protect mice passively against infection with *Trypanosoma gambiense*[10]. Thus, the residual immunity, however slight, seems to have as great a bearing on the outcome of infection as does the profound immunosuppression. This point may be relevant to the wide spectrum of infectious diseases for which immunodepression has been described.

A relationship between variable antigenic type and panosome virulence was first proposed by McNeillage and Herbert[11]. In the present study, although clones NIM 2 and 6 are antigenic variants or subclones derived from a common strain, they differ slightly with respect to both virulence and immunosuppressive activity. The possibility that the variant antigens are themselves responsible for the suppression is now being investigated.

Apart from the intrinsic suppressor activities of *T. brucei* strains, the level of parasitaemia (suppressor dose) undoubtedly contributes to the overall pattern of secondary immunodeficiency. This and previous studies[2] have generally noted an inverse relationship between the survival time and parasite load during the first peak of parasitaemia. However, the level of initial parasitaemia may itself reflect the degree of suppression induced by trypanosome growth early in infection. This is only true for those clones which have comparable replication rates (for example, NIM 2, 6 and 7), which must therefore establish different levels of growth as a consequence of host immune parameters.

Antigenic variation seems to be the principal mechanism whereby the survival of African trypanosomes is prolonged in the host. Depression of anti-trypanosome responses might be required to prevent rapid, complete elimination of the parasite before antigenic variants are expressed. On the other hand, at least partial responsiveness is required to prevent one variant from growing in an uncontrolled fashion, a condition which would also preclude expression of future variants. If the goal is an overall, non-qualitative depression of responsiveness to a residual level, then IgG responses could be affected first because they are more susceptible to general mechanisms of suppression,

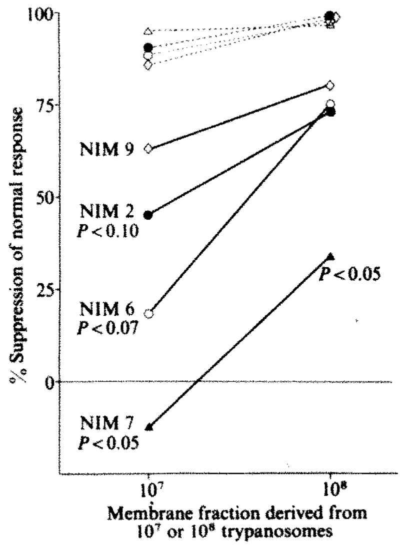

Fig. 2 Comparative suppression of SRBC response by membrane fractions derived from four clones of *T.b. brucei*. Crude membrane fractions from 10^8 or 10^7 trypanosomes were prepared as previously described[8]. Briefly, freeze-thawed homogenates of each clone were spun at 12,000g to remove nuclei, kinetoplasts and large debris. Membrane fractions were collected by high speed centrifugation (150,000g for 90 min), suspended in phosphate-buffered saline and injected intraperitoneally into (CBA/H$\times$C57BL/6)F_1 female mice. Mice were primed 4 d later with 4×10^7 SRBC, and the direct and indirect anti-SRBC response assayed 6 d after priming by the slide modification of the Jerne technique[14]. Each point is the arithmetic mean (five mice per group) of the per cent suppression of the normal response. ——, Direct (IgM) PFC; - - - - - -, indirect PFC developed using a rabbit antiserum specific for mouse IgG. P values were ascertained by Student's t-test for comparison of each mean value with the corresponding next greater mean. Statistic is omitted when no significant difference between the respective mean values was found.

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n of immune response to SRBC after infection with different clones of *T.b. brucei*

no.	clone	Direct PFC	% Normal	Indirect PFC	% Normal
1	—	53,000 ± 8,400		169,000 ± 25,000	
	NIM 6	1,200 ± 2,100*	2	<500	<1
	NIM 2	900 ± 1,300*	1	< 500	<1
2	—	84,000 ± 10,400		143,000 ± 33,000	
	NIM 7	14,200 ± 5,100	17	600 ± 420	<1

Mice were infected with 200 parasites and primed with 4×10^7 SRBC 10 d later. Assay of direct PFC on day 4 and indirect PFC on day 6 were as in Fig. 2; values represent the arithmetic mean ± s.d.; four to five mice per group.

* Value not significantly different from background PFC of unprimed, infected mice.

including disturbances in lymphoid function at the level of B and T cells and macrophages. Alternatively, selective inhibition of IgG as opposed to IgM responses might be purposeful and advantageous to parasite survival. Terry has suggested that although IgM antibody is effective in the control of circulating trypanosomes, it is less effective than IgG classes in reaching tissue parasites from which antigenic variants are thought to arise[1]. The present data are strikingly consistent with this interpretation. Complete suppression of IgG responses and only partial suppression of IgM seems to ensure chronicity of infection, and the intrinsic differences between *T. brucei* clones in suppressing IgM responses seem to form a basis for parasite virulence.

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Biochemical basis for the continuous copulation of female *Schistosoma mansoni*

Kristine H. Atkinson & Burr G. Atkinson

Department of Zoology, University of Western Ontario, London, Ontario, Canada N6A 5B7

Sexual dimorphism in blood flukes of the family Schistosomatidae is marked, and until the male clasps the female in the gynaecophoric canal the female cannot attain sexual maturity[1] or migrate out of the liver[2]. When previously-paired egg-producing females are transferred to a new host without males, the vitelline cells necessary for egg production degenerate[3]. By means of sensitive fluorographic analysis of electrophoresed polypeptides, we have now found molecular evidence of an obligatory continuous relationship between the sexes in *Schistosoma mansoni*. The male worm retains little of the protein it produces in greatest abundance, and this protein is electrophoretically identical to the most abundant protein found in, but not synthesised by, the female.

To detect which proteins were actually produced by the worms, *S. mansoni* of the NIH strain were perfused from C57BL/6B mice at 70 days post-infection, mechanically separated according to sex, and incubated for 24 h in medium HB597 (Connaught) with 10% fetal calf serum and 8 μCi ml^{-1} ^{14}C-leucine (Specific activity 339 mCi mmol^{-1}; NEN). The worms were washed in saline and homogenised in 2% SDS and 5% β-mercaptoethanol in 80 mM Tris-HCl (*p*H 6.8), heated at 100 °C for 1 min, cooled and precipitated with 20% trichloroacetic acid (TCA). After centrifugation at 5,000 *g* for 10 min, the pellets were washed twice with cold 5% TCA, heated in 5% TCA at 90 °C for 15 min, washed with ethanol/ether (2:1) saturated with sodium acetate, washed in ether and dried overnight *in vacuo*. The dried protein precipitates were resolubilised in the homogenising solution, the protein determined by TCA-turbidity[4] and radioactivity measured in a Beckman LS-255 liquid scintillation counter. Vertical SDS-polyacrylamide slab gels were prepared by the method of Laemmli[5], and known amounts of radioactivity or protein were added to each well. Standards of known molecular weight (MW), mouse serum and proteins of the *in vitro* culture medium were co-electrophoresed. After photography of the Coomassie blue staining patterns, the gels were prepared for fluorography[6,7], wherein the dried gel containing isotope-labelled peptides is apposed to pre-flashed Kodak X-Omat R X-ray film and exposed at −70 °C. Only newly synthesised polypeptides having incorporated radioactive leucine from the *in vitro* medium show up as bands in the fluorogram. The procedure was also carried out with a strain of *S. mansoni* inbred in the laboratory of R. B. Short (Florida State University), grown in a white mouse host. The results were virtually identical, with only very minor quantitative differences perceivable in both staining and fluorograms. Protein similarity among *S. mansoni* strains was also found immunoelectrophoretically by Capron *et al.*[8].

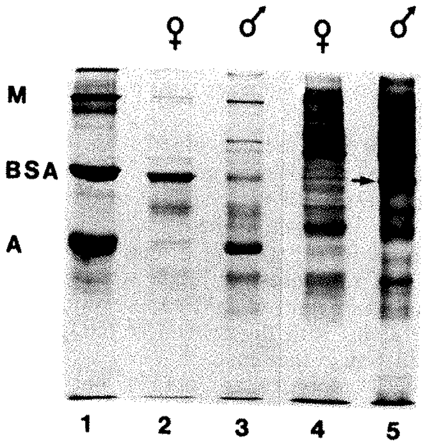

Fig. 1 SDS–8% polyacrylamide gel electrophoretic patterns of the polypeptides of adult male and female *Schistosoma mansoni*. In lane 1 of this composite photograph of a single gel, standard purified polypeptides are stained with Coomassie blue. M = myosin heavy chain, MW 200,000; α = α-actinin, 105,000 MW: BSA = bovine serum albumin, 58,000 MW; A = actin, 43,000 MW; T = tropomyosin, 31,000 MW. The stained patterns of the female (lane 2) and male (lane 3) reveal a high degree of identity, but a prominent band in the female is evident at about 66,000 MW. As equal amounts of protein were placed in these wells, this is evidently a striking quantitative difference in comparison with the faint band in the male. Lanes 4 and 5 are photographs of the fluorograms exposed by the same samples shown stained in lanes 2 and 3. Equal amounts of radioactivity were placed in the wells, and these autoradiographs show which proteins were synthesised by isolated worms *in vitro*. That the females do not produce their most prominent stained band is striking. The males, on the other band, actively produce a polypeptide at this MW of which they have only small quantities, as seen stained in lane 3.

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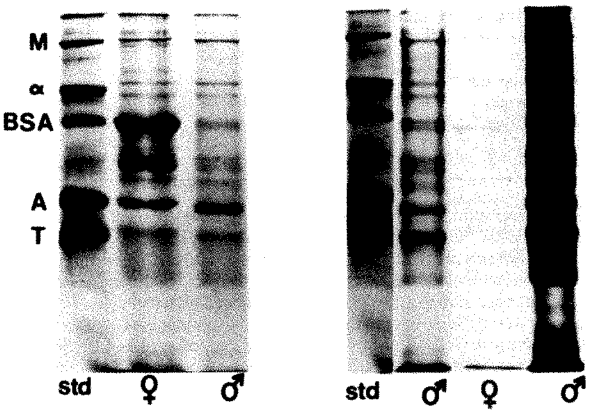

Fig. 2 SDS – 10% polyacrylamide gel electrophoretic patterns of unlabelled female *S. mansoni* after remaining *in copula* with labelled males for 20 h. On the left are the Coomassie blue staining patterns of the samples shown in the three fluorograms of the composite picture on the right (the standards are a stained preparation). The male and female samples on the far right are a comparison exposure to show the low radioactivity profile of the females; the male fluorographic pattern next to the standard was prepared with a shorter exposure. The 66,000-MW protein is picked up by the female in greater amounts than any other. The other proteins supplied to the female by the male mirror his most actively synthesised proteins. Prolonged exposure of the female samples suggested a quantitative synthetic pattern typical of the male. (Abbreviations as in Fig. 1.)

Figure 1 is a composite of the Coomassie blue patterns and fluorograms of the total proteins of male and female *S. mansoni*. In the female, the heavily stained band with a mobility slightly faster than that of bovine serum albumin (BSA) is outstanding. Its absence in the fluorogram of the same gel means that this protein could not have been synthesised by the female. Two-dimensional electrophoretic analysis of this protein indicates that its MW and isoelectric point do not match any of the proteins found in the culture medium or the mouse serum, and so this does not represent a 'captured' protein.

The absence of a major protein synthesised uniquely by mature females was at first disappointing, as it was expected that the commitment to egg and vitelline cell production would be reflected in the proteins produced. However, quantitative differences in the amount of a protein produced by the male (arrow, Fig. 1) but found in relatively low amounts (revealed by the staining pattern) compared with the female pointed to the transport of this substance transtegumentally between the sexes. The fact that the female is almost entirely enveloped by the male tegument and that the male gynaecophoric canal has a modified surface[9] supported this view.

To characterise further the nature of the 66,000-MW protein in question, the experiment was repeated with males labelled for 24 h, then rinsed and mated to unlabelled females for 20 h. Figure 2 depicts the fluorogram developed after electrophoresis of the polypeptides of the females following removal of the males and three washes with fresh medium. Transfer of the labelled polypeptide from the male to the female within 20 h was significant; the females also picked up some minor common polypeptides. No radioactivity was detectable in the TCA-soluble fractions from the homogenised females, indicating the absence of free ^{14}C-leucine. The medium in which the mated worms had been incubated contained negligible amounts of radioactivity (<30 c.p.m. μl^{-1}), so it is very unlikely after the thorough rinsing that the labelled polypeptides revealed in the fluorogram were a result of passive transfer. The concept of direct transtegumental transport between the sexes is supported by the studies of Shaw *et al.*[10], who found that the vitelline cells of immature females were stimulated to develop by extracts of whole male worms, but not by the medium in which the males were cultured. It is unlikely that the protein is transferred with sperm, as in butterflies[11], because the seminal receptacle is o about 0.1% of the female's volume, whereas the 66,000-M band is about one-tenth of her total stained protein.

Our subsequent work with two-dimensional electrophoresis and fluorographic analysis (manuscript in preparation) has shown that this protein represents the major difference between the male and female in terms of their acid-precipitable proteins. Ruppel and Cioli[12] have published a polyacrylamide gel electrophoresis staining pattern with similar results, although they do not discuss this protein with a mobility slightly faster than BSA.

The absence of parthenogenesis in the genus *Schistosoma* (except for *Schistosoma mattheei*[13]) seems predictable in the light of this molecular symbiosis maintained by the sexes for the production of offspring. A cure for schistosomiasis may consist of finding a method of keeping the sexes unpaired.

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The *in vitro* transmission frequency of the t^6 allele

James McGrath & Nina Hillman

Department of Biology, Temple University, Philadelphia,
Pennsylvania 19122

The *T* region, located on chromosome 17 in the mouse, is a complex genetic locus which includes a series of recessive mutations, t^n (refs 1, 2). These mutations include several alleles (also referred to as *t*-haplotypes[2]) which are embryonic lethals (t^L). An additional phenotypic expression of the recessive lethal mutations is a distorted transmission of the *t* allele by the heterozygous male parent. Most T/t^L and $+/t^L$ males transmit the recessive mutation to over 50% of their offspring. Two conditions are known to modify significantly the transmission frequency of some t^L alleles: the length of time between insemination and fertilisation[3–5] and the genetic background of the female[6]. The present studies were undertaken to determine if the distorted transmission frequency of one of the lethal alleles, t^6, could be reproduced *in vitro*. The data show that the *in vitro* transmission frequency of the t^6 mutation is non-mendelian and is not significantly different from the frequency of transmission of this mutation following *in vivo* delayed matings. The *in vitro* frequency is, however, significantly lower than the normal mating frequency and does not vary as a function of incubation time. These data suggest that the observed delayed and normal mating frequency differences result from time-dependent interactions between the t^6-bearing spermatozoon and the female reproductive tract.

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1 Normal and delayed transmission frequencies of the t^6 allele determined by the *in vitro* culture of one-cell embryos fertilised *in vivo*

	Control cross $T/+$♀ × $+/t^6$♂		Experimental cross $+/t^6$♀ × $+/t^6$♂		
	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	Transmission frequency*
Normal mating	409	0	724	187	0.52
Delayed mating	281	0	481	58	0.24

(C57BL/6J♀ × T/t^6♂) F_1 females were injected intraperitoneally with pregnant mare serum gonadotropin (5 IU) followed 48 h later by an injection of human chorionic gonadotropin (HCG; 5 IU)[25]. The females were caged with $+/t^6$ [(BALB/c♀ × T/t^6♂)F_1] males and on the following day the pregnant females were killed by cervical dislocation. One-cell embryos were flushed from the extirpated oviducts and placed in modified Whitten's medium[8,26] (4 mg ml^{-1} bovine serum albumin, Miles; 75 μM Na_2EDTA) under silicone oil (Dow Corning 200 Fluid, 50 cSt viscosity). The silicone oil (equilibrated with modified Whitten's medium, 7:1 v/v) and modified Whitten's medium were gassed with 5% O_2, 5% CO_2 and 90% N_2 before use. For the first two cleavage divisions the embryos were cultured in a sealed anaerobic jar (Torbal) which had been gassed for 20 min with 5% O_2, 5% CO_2 and 90% N_2. At the four-cell stage the dishes containing the embryos were transferred to an incubator with a continuous gas flow of 5% CO_2 in air. Blastocyst-stage embryos were transferred to a 35 × 10 mm tissue culture dish (Falcon Plastics) containing 2 ml of modified Eagle's medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum (GIBCO)[9]. The dishes were returned to the continuous gas flow incubator for an additional 4 days. Outgrowths were scored as mutant or non-mutant using a Zeiss inverted microscope equipped with phase contrast optics (×160) on days 8 and 9 post-fertilisation. Preimplantation development at normal mating times from the two-cell stage to the blastocyst stage for the control and experimental crosses was 456/486 (94%) and 814/872 (93%), respectively, and at delayed mating times 311/321 (97%) and 531/546 (97%), respectively. Outgrowth of the blastocysts at normal mating times for the control and experimental crosses was 409/447 (91%) and 724/798 (91%), respectively, and delayed mating times 281/310 (91%) and 481/528 (91%), respectively. Significant differences were computed using a χ^2 contingency test. For normal matings, females were caged with males immediately after the HCG injection. For delayed mating, females were caged with males 12 h after the HCG injection.

* Transmission frequency = 2 × (no. of t^6/t^6 outgrowths/total no. of outgrowths).

These studies were possible because: (1) ova from F_1 hybrid females fertilised either *in vitro*[7] or *in vivo*[8] can develop to the blastocyst stage when incubated in modified Krebs–Ringer medium; (2) these blastocysts will continue to develop ('outgrow') when placed in modified Eagle's medium[9]; (3) the outgrowths from t^6/t^6 uterine blastocyst embryos can be distinguished morphologically from their phenotypically wild-type littermates[10–12]; (4) the number of mutant outgrowths in these genetically mixed embryo populations is equivalent to the expected number based on the tested transmission frequency of the t^6 allele[11]. Mutant outgrowths developing *in vitro* from uterine blastocysts can be distinguished from their normal counterparts by the failure of their trophoblast cells to polyploidise and become giant and by a reduced viability of the inner cell mass cells. However, only the former criterion could be used in the present studies because outgrowths developing from ova fertilised *in vitro* may not contain an inner cell mass.

To ensure the development of embryos in culture following *in vitro* fertilisation, ova from hybrid females were used[13,14]. These females were obtained by crossing C57BL/6J females with T/t^6 males. The offspring from this cross are phenotypically distinguishable: $+/t^6$ females have tails of normal length whereas $T/+$ females are short-tailed. Ova from the $T/+$ females were used as the controls for the *in vitro* fertilisation studies.

Before using spermatozoa from $+/t^6$ males for *in vitro* inseminations, we tested the males for their transmission frequencies in normal and delayed matings with the hybrid $+/t^6$ females. Following normal matings, spermatozoa reside in the uterus for 6–7 h before ovulation and fertilisation. In delayed matings, fertilisation is initiated 1–2 h post-insemination[15]. After both normal and delayed matings, the fertilised one-cell embryos were removed from the female and placed in culture. Blastocyst outgrowths were scored to distinguish the t^6 homozygotes. Table 1 gives the percentage transmission of the t^6 allele from $+/t^6$ males following both normal and delayed matings with hybrid $+/t^6$ females. The data show that in normal

Table 2 *In vitro* transmission frequency of the t^6 allele

	*In vivo**		*In vitro*				
	Transmission frequency†		Control cross $T/+$♀ × $+/t^6$♂		Experimental cross $+/t^6$♀ × $+/t^6$♂		
Male no.	Normal mating	Delayed mating	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	Transmission frequency†
1	0.70 (43)‡	—	21	0	96	12	0.25
2	0.52 (66)	—	108	0	87	6	0.14
3	0.41 (58)	—	68	0	108	14	0.26
4	—	0.16 (49)	90	0	78	8	0.21
5	—	0.18 (46)	37	0	68	6	0.18
6	—	0.38 (47)	43	0	56	8	0.29
		Total	367	0	493	54	0.22

(C57BL/6J♀ × T/t^6♂)F_1 females were injected as described in Table 1 legend. At 12–13 h after the HCG injection the females were killed and their oviducts excised and placed in drops of modified Tyrode's medium[27] under silicone oil. The cumulus masses containing the ova were removed from the ampullary regions of the oviducts and twice transferred to fresh drops of medium (0.2 ml) under silicone oil. Spermatozoa were obtained from the cauda epididymides and vasa deferentia excised from (BALB/c♀ × T/t^6♂)F_1 males. The ducts, placed into 1 ml of modified Tyrode's medium under silicone oil, were minced and the spermatozoa allowed to disperse for 20 min. A 100-μl sample of the spermatozoan suspension was diluted approximately 1:3 with modified Tyrode's medium. A 10-μl aliquot of the diluted suspension was added to the 0.2-ml drops of medium containing the ova. Final spermatozoan concentrations ranged from 1.3 to 2.7 × 10^5 spermatozoa per ml. The insemination dishes were placed in an anaerobic jar and gassed for 20 min with 5% O_2, 5% CO_2 and 90% N_2 at 37 °C. The jar was subsequently sealed and the gametes were allowed to co-incubate for 6 h. After this 6-h period, the ova were removed and washed through four drops of modified Whitten's medium. Preparation of the fertilisation medium, culture medium, silicone oil and the culture of the embryos were as described in Table 1 legend. The procedures for *in vitro* fertilisation followed the protocol of Fraser and Drury[27] except that the sodium chloride concentration in the modified Tyrode's medium was reduced to 85.2 mM. Fertilisation, based on the number of one-cell ova that cleaved to the two-cell stage, averaged 86% (75–96% range). Preimplantation development from the two-cell stage to the blastocyst stage for the control and experimental crosses was 446/511 (87%) and 565/653 (87%), respectively. The number of blastocyst outgrowths/total number of blastocysts from the control and experimental crosses was 367/417 (88%) and 493/554 (89%), respectively.

* Individual males, before being used for the *in vitro* fertilisation experiments, were tested *in vivo* for their transmission frequencies at either normal or delayed mating times. The times at which the animals were mated and the subsequent culture of the one-cell embryos were as described in Table 1 legend.

† Transmission frequency = 2 × (no. of t^6/t^6 outgrowths/total no. of outgrowths).

‡ Number in parentheses refers to the number of outgrowths on which the *in vivo* transmission frequency value is based.

Table 3 Effect of *in vitro* incubation period on *in vitro* transmission frequency of the t^6 allele

	*In vivo**		*In vitro*					
	Transmission frequency†			0 h			6 h	
Male no.	Normal mating	Delayed mating	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	Transmission frequency†	No. of blastocyst outgrowths	No. of outgrowths with t^6/t^6 phenotype	Transmission frequency†
7	0.38 (52)‡	0.00 (29)	87	7	0.16	89	8	0.18
8	—	—	100	7	0.14	43	4	0.19
9	0.60 (73)	0.28 (36)	72	6	0.17	41	5	0.24
10	0.40 (45)	0.09 (45)	58	9	0.31	75	4	0.11
		Total	317	29	0.18	248	21	0.17

Females were injected so that recently ovulated oocytes were available at 0 and 6 h. The gametes were obtained from (C57BL/6J♀ × T/t^6♂)F_1 females and (BALB/c♀ × T/t^6♂)F_1 males as described in Table 2 legend. The 0-h data list the results from inseminations with diluted spermatozoan suspensions mixed with ova immediately after the 20-min dispersal period. During the 6-h *in vitro* incubation period the spermatozoa were maintained in 1 ml of modified Tyrode's medium at 37 °C in a gas phase of 5% O_2, 5% CO_2 and 90% N_2. An aliquot of the spermatozoan suspension was then freshly diluted and added to the second group of oocytes. Spermatozoan concentrations ranged from 2.1 to 4.3×10^5 spermatozoa per ml.

* Individual males, before being used for the *in vitro* fertilisation experiments, were tested *in vivo* for their transmission frequencies at either normal or delayed mating times. The times at which the animals were mated and the subsequent culture of the one-cell embryos were as described in Table 1 legend.

† Transmission frequency = 2 × (no. of t^6/t^6 outgrowths/total no. of outgrowths).

‡ Number in parentheses refers to the number of outgrowths on which the *in vivo* transmission frequency value is based.

matings the transmission frequency of the t^6 allele is mendelian (0.52) and that the transmission frequency of the t^6 allele is significantly reduced (0.24) if the matings are delayed until the time of ovulation ($\chi^2 = 33.0$; $P < 0.0001$). These findings support not only the earlier observation that the length of time between insemination and fertilisation affects the transmission frequency of the t^6 allele[16], but also the observation that the genotype of the female can modify the transmission frequency of certain t^L alleles. Males which are heterozygous for the t^6 allele typically transmit the allele at a frequency of greater than 70% in matings with BALB/c (ref. 17) and SWR/J (ref. 11) mice. This is significantly higher than the frequency of transmission of this allele in matings with either the hybrid females or C57BL/6J females. In the latter matings ($T/t^6 \times$ C57BL/6J), the transmission frequency (0.46) was determined from the number of normal-tailed offspring among the total number of progeny (1,070/2,333).

For the *in vitro* fertilisation studies, ova from hybrid $T/+$ and $+/t^6$ females were fertilised *in vitro* by spermatozoa from $+/t^6$ males. Aliquots of spermatozoa from the same male were used to fertilise oviducal ova from females of both genotypes. The results (Table 2) show that all the 367 outgrowths developing from fertilised $T/+$ ova developed normally and all exhibited the wild-type phenotype. Of the ova obtained from $+/t^6$ females and fertilised *in vitro*, 54 of the 493 outgrowths were phenotypically abnormal and were scored as homozygous mutant embryos. Thus, the transmission frequency of the t^6 allele *in vitro* is 0.22. This frequency is significantly less than the frequency observed during *in vivo* normal matings ($\chi^2 = 39.9$; $P < 0.0001$). Conversely, the difference between the *in vitro* transmission frequency and the *in vivo* delayed frequency is not significant ($\chi^2 = 0.19$; $P > 0.05$).

In vitro, fertilisation begins 1–2 h after insemination[18–20]. During this period, the spermatozoa undergo capacitation and penetrate the investments surrounding the ovum. In delayed matings the spermatozoa also reside in the female reproductive tract, before fertilisation, for 1–2 h (ref. 15). In normal matings, however, the time between insemination and fertilisation is considerably longer.

Because *in vitro* insemination and *in vivo* delayed insemination result in similar reductions in the transmission frequency of the t^6 allele, and because both procedures are associated with reduced time between insemination and fertilisation, we tested the affect of 'time *in vitro*' on the frequency of t^6-allele transmission. We compared the transmission frequency of this allele by using aliquots of gametes from the same male to inseminate ova following either a 0 or 6 h incubation period of the spermatozoa. The results (Table 3) show that the increased period of incubation does not increase the *in vitro* transmission frequency of the t^6 allele (0.18 versus 0.17; $\chi^2 = 0.018$; $P > 0.05$).

The data from these latter studies disprove the hypotheses that the distorted transmission frequency of a t-allele is caused by either a selective death of the non-t-bearing spermatozoa with time[21,22] or a differential transport of the + - and t-bearing gametes through the uterotubal junction[23,24]. Our findings suggest, rather, that the increase in transmission frequencies between delayed and normal matings is dependent on an interaction between the female reproductive tract and the male gamete, and that this interaction is time dependent. As the transmission frequency of the tested allele can be significantly affected by the genotype of the female, it is probable that the enhancing factor is intrinsic to the uterine and oviducal environments and is variable among the females of different genotypes.

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for a role of plasma kallikrein in the activation of prorenin

K. Wolfgang Rumpf, Klaus Becker, Ulrich Kreusch, Sigrid Schmidt, Roland Vetter & Fritz Scheler

Department of Medicine, Division of Nephrology, University of Göttingen, Robert-Koch-Str. 40, 3400 Göttingen, FRG

The proteolytic enzyme renin seems to play an important part in the humoral control of blood pressure by initiating a reaction which finally leads to the production of the vasoconstrictor peptide angiotensin II. However, it has recently been shown that normal human plasma contains a higher molecular weight form of renin (prorenin), which is enzymatically inactive[1–6]. *In vitro*, prorenin is converted to the active renin enzyme by various unphysiological procedures such as cryoactivation[7–9], acid activation[1,6,7,9] or the addition of trypsin[10,11] or pepsin[11,12], the activation by trypsin being equal to that by acidification[13–15]. Although the physiological role of prorenin remains unclear, it has been shown that differences exist in the response to stimuli between prorenin and the active renin enzyme[3,6,7]. Recently, it has been shown that serine protease(s) may be involved in the activation process of prorenin, during both cryoactivation[7,16] and acid activation[7]. Furthermore, human urinary kallikrein added to plasma in low concentrations is able to activate prorenin completely *in vitro*[17]. Therefore, it has been speculated that urinary kallikrein might be the physiological *in vivo* activator of prorenin[17–19]. This also seemed plausible because renal kallikrein is produced in cells adjacent to the renin-producing cells[20]. On the other hand, plasma kallikrein has been implicated in the activation of prorenin[15,21–23]. In the experiments described below we tried to obtain some more direct evidence for the *in vivo* role of kallikrein(s) in the prorenin activation. Our results suggest that plasma kallikrein may indeed be a physiological activator of prorenin, but they do not support a role for urinary (renal) kallikrein in the activation of prorenin.

If kallikrein is the *in vivo* activator of prorenin, a negative correlation between kallikrein (urinary or plasma) and the proportion of prorenin (prorenin/total renin) may be expected. In 13 normal human volunteers, urinary kallikrein excretion and plasma prekallikrein concentration were correlated to the proportion of prorenin in plasma in different states of sodium balance, which is known to have a marked effect on urinary kallikrein excretion. Urinary kallikrein[24] and plasma prekallikrein[24,25] were measured using chromogenic tripeptide substrates (substrates S2266 and S2302, Kabi Diagnostica). Active renin and prorenin were determined by radioimmunoassay of angiotensin I using acid activation and nephrectomised sheep plasma as renin substrate[6].

Whereas sodium excretion was significantly reduced during sodium restriction (diet containing approximately 1.0 g NaCl per day) and, concomitantly, urinary kallikrein excretion increased fourfold, plasma prekallikrein concentration and the proportion of prorenin remained essentially unchanged (Table 1). During sodium loading (*ad libitum* diet plus 7.5 g NaCl per day for 3 days) sodium excretion was significantly higher than during the control period. Urinary kallikrein excretion was still elevated, presumably because of a significantly elevated urinary volume. Plasma prekallikrein and the proportion of prorenin did not change significantly during sodium loading.

Figure 1 shows that during all experimental periods, there was no correlation between urinary kallikrein excretion and the proportion of prorenin. This is in contrast to the results of other authors, who found a weak correlation between these two parameters[19]. In our experiments, in spite of profound alterations in urinary kallikrein excretion, the proportion of prorenin remained unchanged. This certainly does not exclude a role of urinary kallikrein in the activation of prorenin, but does make it rather improbable.

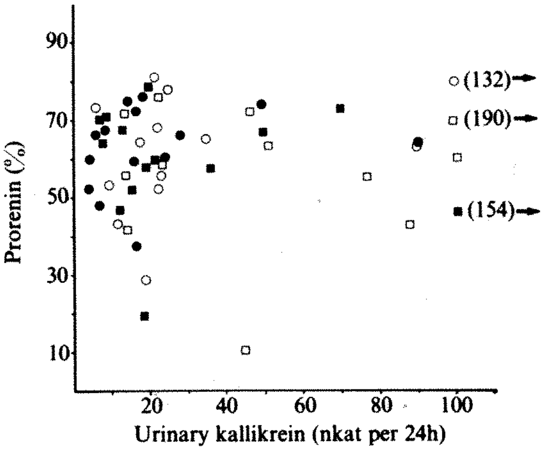

Fig. 1 Urinary kallikrein excretion and proportion of prorenin in different states of sodium balance in 13 normal human volunteers. ●, Normal diet; ○, sodium loading; ■, sodium restriction (2 days); □, sodium restriction (4 days).

Although during the different experimental periods plasma prekallikrein did not change significantly (Table 1), a close relationship between plasma prekallikrein concentration and the proportion of prorenin (Fig. 2) was observed: the higher the plasma prekallikrein, the lower the proportion of prorenin in plasma. The linear correlation coefficients were significant during all periods of the experimental protocol (*ad libitum* diet: $y = 96.8 - 0.64x$; $r = 0.71$; $n = 13$; $P < 0.01$. Sodium restriction: $y = 102.9 - 0.85x$; $r = 0.70$; $n = 22$; $P < 0.001$. Sodium loading: $y = 97.5 - 0.69x$; $r = 0.60$; $n = 13$; $P < 0.05$).

There are two possible explanations for the correlation. First, that this is an *in vitro* effect, which is due to incomplete activation of prorenin by the acidification procedure. However, this seems

Table 1 Urinary volume, sodium excretion, urinary kallikrein excretion, plasma prekallikrein and proportion of prorenin in different states of sodium balance

	ad libitum diet	Days of sodium restriction				Sodium loading
		1	2	3	4	
Urinary volume (ml per 24 h)	1,316 ± 143	1,551 ± 183	1,418 ± 175	1,343 ± 176	1,338 ± 113	1,916 ± 264†
Sodium excretion (mmol per 24 h)	170.0 ± 19.8	124.8 ± 14.8‡	48.9 ± 4.8§	27.7 ± 4.1†	16.4 ± 1.9§	331.7 ± 31.3§
Urinary kallikrein excretion (nkat per 24 h)	16.4 ± 3.4	21.3 ± 4.4‡	22.9 ± 5.1†	50.8 ± 12.1‡	64.6 ± 15.6‡	33.2 ± 10.0*
Plasma prekallikrein (μkat l^{-1})	53.2 ± 3.6		48.6 ± 3.6		52.3 ± 5.0	51.4 ± 3.6
Proportion of prorenin (%)	62.7 ± 3.2		60.5 ± 4.2		57.1 ± 4.7	61.0 ± 4.5

Values are given as means ± s.e.m., $n = 13$. Significantly different from controls on an *ad libitum* diet: *$P < 0.05$; †$P < 0.02$; ‡$P < 0.005$; §$P < 0.001$.

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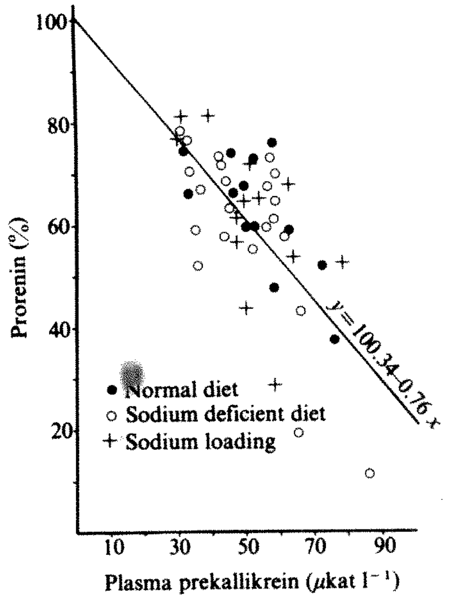

Fig. 2 Correlation between plasma prekallikrein and the proportion of prorenin in 13 normal human volunteers in different states of sodium balance. $r = 0.6652$; $n = 48$; $P < 0.001$.

improbable as it has been repeatedly shown that the so-called 'acid'-activation is complete and equivalent to the activation by trypsin[13-15]. Second, the correlation found may be physiologically significant and is applicable to the *in vivo* situation. This, of course, would be a strong argument in favour of an *in vivo* role of plasma kallikrein in the activation of prorenin.

Interestingly, the intercept on the ordinate in all three groups was near 100%, suggesting that in a plasma free of prekallikrein, renin would only be present in the form of prorenin and active renin enzyme would not be formed in these conditions. This, however, would suggest that plasma kallikrein not only is one of several activators of prorenin, but also that it may be the only activator of physiological significance. However, as our results were obtained in normal subjects only, we cannot exclude the possibility that in certain pathological conditions the relationship demonstrated may not apply.

Thus, our results do not support the concept that urinary kallikrein is of importance in the *in vivo* activation of prorenin, as has been proposed[17-19], but they strengthen the view that plasma kallikrein might be the physiological activator of prorenin[15,21,22].

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Multiple innervation of cerebellar Purkinje cells by climbing fibres in *staggerer* mutant mouse

F. Crepel

Laboratoire de Physiologie comparée, Université Pierre et Marie Curie, F 75230 Paris Cedex 05

N. Delhaye-Bouchaud

Laboratoire de Neurophysiologie ontogénétique, Université Pierre et Marie Curie, F 75230 Paris Cedex 05

J. M. Guastavino & I. Sampaio

Laboratoire d'Ethologie et Sociobiologie, Université Paris XIII, 93000 Bobigny, France

Development of the peripheral nervous system involves an initial overgrowth of synaptic contacts, followed later by the regression of the redundant connections[1-4]. Similarly, in the central nervous system, the adult-type one-to-one relationship between climbing fibres (CFs) and cerebellar Purkinje cells (PCs) is preceded at early developmental stages by a multiple innervation of PCs by CFs[5]. The regression of the supernumerary contacts fails to occur when PCs develop in a cellular environment devoid of granule cells[6-9], suggesting that it may depend on the formation of synapses between parallel fibres and PCs. However, one cannot preclude the possibility that granule cells intervene in the regressive process by a nonsynaptic action, for instance by emitting a chemical signal at the level of their soma, which could act on the CF-PC synapses. In this respect, the recessive *staggerer* (*sg*) mutation[10], is of special interest because one of its major phenotypical expressions in homozygous *sg*/*sg* animals is the selective and almost complete absence of synapse formation between parallel fibres and Purkinje cells, in spite of the presence of an initially important number of granule cells and of parallel fibres during the synaptogenesis period, that is, until about the end of the 3rd post-natal week. Later on, the relay cells progressively die, probably because of the absence of synaptic contacts with PCs[11-13]. This is in marked contrast with the other agranular animals studied so far (X-ray irradiated rats and *weaver* mutant mice[6,7,9]) where granule cells lack from the very beginning[6,9,12]. As the *staggerer* mutation does not prevent the formation of functional synapses between CFs and PCs[14], it was important to establish whether PCs can reach the adult-type monoinnervation in the mutant. We show here that each PC is contacted by several CFs in the adult homozygous *staggerer* mouse, thus supporting the first hypothesis.

Hybrids of C57BL and DBA2J mice originating from the Jackson Laboratory and bearing the mutation were raised at the Laboratory and homozygous mutants *sg*/*sg* were obtained by intercrossing heterozygous animals. Mutant mice were distinguished from their littermates by clinical features[12] and they were kept alive after weaning by the use of appropriate feeding conditions[15]. The organisation of the cerebellar cortex at the ultrastructural level in adult *sg*/*sg* animals was identical to that previously described (C. Sotelo, personal communication) and they were used for electrophysiological studies 5 to 7 months after birth. After initial ether or Nembutal (30 mg per kg intraperitoneally) anaesthesia, animals were paralysed,

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spired, and the vermis was exposed. Juxtafastigial ion was performed with fine bipolar electrodes and sites were confirmed as previously reported[14]. recordings from PCs were obtained with glass micropipettes (d.c. resistances between 10 and 15 MΩ). An active bridge circuit was used to pass current through the recording microelectrode. The position of the tip of the microelectrode in the cerebellar cortex was determined by iontophoretic injection of methyl blue.

Twenty four PCs identified before cell penetration by their antidromic activation[14,16] elicited by JF stimulation (data not shown) were impaled and their electrical activities were analysed. In all the recorded cells, the orthodromic responses evoked by JF stimulation closely resembled a CF response[16]: they consisted of an initial full spike followed by a prolonged plateau of depolarisation on which partial spikes were or not superimposed (Fig. 1*ai*). Cell deterioration led rapidly to a suppression of spike potentials within the response and the underlying excitatory postsynaptic potential (e.p.s.p.) was revealed (Fig. 1*aii*). It had the typical time course of a CF-e.p.s.p.[16] but it was graded by steps (2 to 4) when increasing stimulation strength as it is usually observed in multiply innervated PCs[6,9] (Fig. 1*aii*). This type of CF-like e.p.s.p. also occurred spontaneously and it fluctuated in size in a stepwise manner (Fig. 1*aiii*), with a firing rate for each component of the response in the range of CF-mediated e.p.s.ps (1–2 s^{-1}). Moreover, harmaline injection (50 mg per kg intraperitoneally) induced a rhythmic bursting (5 to 10 s^{-1}) of these compound e.p.s.ps (Fig. 1*bi*, ii), confirming they were mediated via CF[17]. Finally, these responses were reversed by depolarising currents injected through the microelectrode (Fig. 2), as expected for chemically mediated synaptic potentials[18], and current values for reversal were very similar (10–15 nA) to those found for normal CF-e.p.s.ps[19] and for graded CF-e.p.s.ps in adult X-ray irradiated rats[9].

It seems unlikely that these graded CF-like activities could be mediated through the mossy fibre-granule cell pathway since at the age studied, almost all granule cells have degenerated in the mutant and since maximum stimulation of the residual parallel fibres either evoked no response or evoked only a single spike in the PCs tested (unpublished observations). The rare parallel fibre-PC synapses which still exist in the mutant[13] could nevertheless be responsible for the late and small e.p.s.ps (as compared to the CF-like response) which were sometimes evoked by JF stimulation in the recorded cells (Fig. 1*aii*, arrow) and which spontaneously occurred at a high firing rate (20–50 s^{-1}) during the resting discharge of PCs, thus corresponding to the simple spike spontaneous firing recorded before cell penetration.

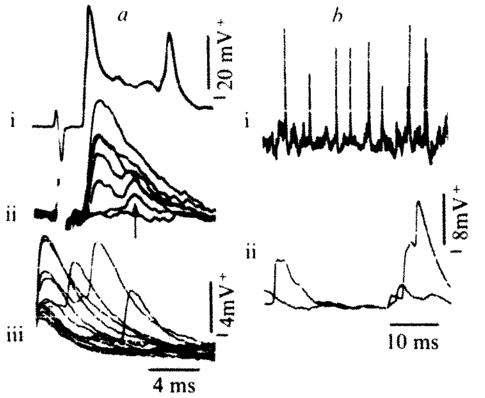

Fig. 1 Intracellular recordings from Purkinje cells. Body temperature was kept constant at 37 °C and the cerebellar vermis was covered with 4% agar in 10% sucrose solution. Resting potentials were −45 mV for the cell illustrated in *a*, and −35 mV for that in *b*. Cells generally remained in a good condition during intracellular recordings for up to 10 min. *ai*, Climbing fibre-like response evoked by JF stimulation; *aii*, same cell as in *ai* after cell deterioration. The underlying e.p.s.p. was graded in a stepwise manner with the intensity of the JF stimulation. *aiii*, Spontaneous climbing fibre-like e.p.s.ps recorded from the same cell as in *ai* and ii. Each sweep was triggered by the rising edge of the response. *bi*, Rhythmical bursting of climbing fibre-like e.p.s.ps at about 5 s^{-1} in another PC after harmaline injection; *bii*, same cell as in *bi* but with a faster sweep speed.

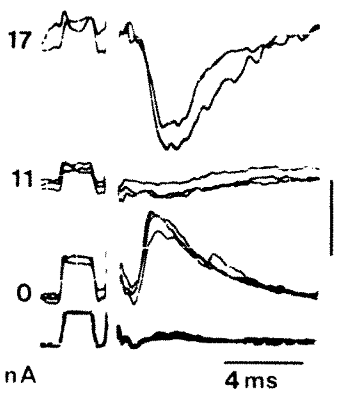

Fig. 2 Reversal properties of a climbing fibre-like e.p.s.p. evoked in a PC by JF stimulation under depolarising d.c. currents. The resting potential of the cell was −42 mV. Current injections were done when the spike generating mechanism of the cell was completely abolished. Ten suprathreshold JF stimulations at 0.5 s^{-1} were applied for each current step (current values are indicated on the left of each record), and the size and polarity of the responses were determined by comparison with the extracellular field potential after withdrawal of the microelectrode (bottom trace). The calibrating pulse on the left of each trace is 2 mV in amplitude and 2 ms in duration.

The present study fully confirms the functional linkage between CFs and PCs in the *staggerer* mouse and establishes that each PC receives several independent CFs in the adult. These results allow the extracellular graded responses evoked in 3-week-old animals by JF stimulation[14] and rhythmically triggered by harmaline injection to be now tentatively re-interpreted as immature CF-responses[20,21] recorded from multiply innervated PCs whose maturation was very delayed[22]. At this early stage a significant number of granule cells is still present and the most striking abnormality is a selective absence of synapses between parallel fibres and PCs[13]. It therefore seems likely that in normal animals, the formation of parallel fibre–PC synapses is responsible for the regression of supernumerary CF-PC synapses which occurs during development. The present study strengthens the view that the *staggerer* mutation does not simply consist of a general reduction in the ability of PCs to form synapses with their afferents (as PCs are multiply innervated by CF) but that the loss of synaptic competence is highly selective.

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Imidazole inhibits a temperature-dependent component of mammalian skeletal muscle action potential

D. W. Cheung* & E. E. Daniel

Department of Neurosciences, McMaster University, Hamilton,
Ontario, Canada L8S 4J9

Temperature-dependent transition of conductance in ionic channels has been described in several membrane systems[1–3]. We report here an abrupt change in the maximal rate of rise of the action potentials of the rat extensor digitorum longus (EDL) at 32 °C, indicating alteration in the functional characteristics of the sodium channels. Tetrodotoxin (TTX) and imidazole were used to investigate further the change in properties of the sodium channels with respect to temperature. TTX is known for its specificity in blocking sodium channels[4]. It has been proposed that the guanidinium group of the TTX molecule is essential for its activity. Other guanidinium compounds have also been shown to be effective in blocking the sodium channels[5]. In this study, imidazole, a compound structurally similar to guanidine, was also tested. We find that TTX and imidazole affect the maximal rate of rise of the action potential in a temperature-dependent manner.

Extensor digitorum longus muscles from male Wistar rats were mounted in a muscle bath and perfused with oxygenated Ringer's solution. The temperature of the bath was regulated by water circulating around its jacket. Intracellular recordings were made with microelectrodes filled with 3 M KCl. The muscles were electrically stimulated with a pair of external platinum wire electrodes. The voltage change was recorded through a WPI M4A electrometer and the maximal rate of rise of the action potential was obtained by a differentiating circuit. The maximal rate of rise of the action potential was measured because it gives an indication of the sodium conductance increase during an action potential[6]. The muscles were allowed to soak in the solutions containing the drugs for at least 20 min. before recording. At least 30 min was allowed for equilibration for any change of temperature.

The change in temperature, as well as TTX and imidazole, had no effect on the resting membrane potential of the muscle fibres. However, a dramatic change in the maximal rate of rise of the action potential with temperature was observed (Fig. 1). From a steady level of about 200 V s^{-1} at temperatures below 32 °C, the rate increased to about double its value within a 5 °C temperature rise.

To investigate the effect of imidazole and TTX on the maximal rate of rise of the action potential with respect to temperature change, three representative temperatures (25 °C, 30 °C and 35 °C) were chosen for more detailed studies, although results obtained at other temperatures followed the same pattern. Imidazole reduced the rate of rise in a temperature-dependent manner. Within the lower temperature range from 20 to 30 °C, in which the maximal rate of rise did not change with temperature, imidazole had no effect. At temperatures above 32 °C, 2.5×10^{-5} M imidazole reduced the rate of rise to the same level as that at lower temperatures (Fig. 2*c*). Higher concentrations of imidazole (up to 10^{-3} M) were not effective in further reducing the rate of rise beyond that observed at lower temperatures.

TTX reduced the maximal rate of rise of the action potential at all temperatures (Fig. 2*b*). At 3.1×10^{-9} M TTX, the depression of the rate of rise was more conspicuous at 35 °C. It was only at higher concentrations of TTX (1.55×10^{-7} M) that the rate was reduced to the same level at all temperatures. Pretreatment of the muscle with imidazole (2.5×10^{-5} M) abolished the temperature sensitivity of the rate of rise to TTX. The rate was reduced to the same level at all temperatures (Fig. 2*d*). Increasing the TTX concentration resulted only in a concomitant reduction at the different temperatures.

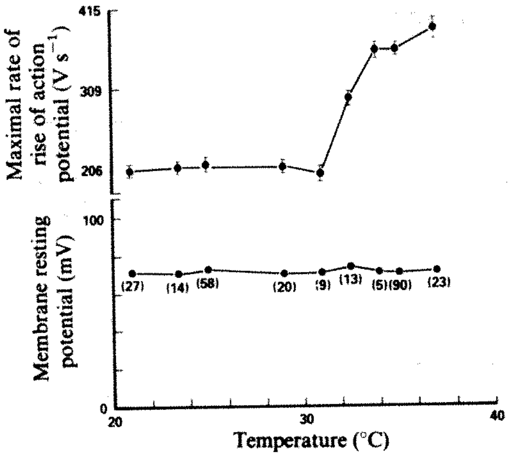

Fig. 1 Effect of temperature change on the maximal rate of rise of the action potential (top) and the resting membrane potential (bottom) of the rat extensor digitorum longus. Although the resting membrane potential was not sensitive to the variation in temperature, the rate of rise became temperature sensitive at around 32 °C, showing a sharp increase with only a small rise in temperature.

The transition of the maximal rate of rise of the action potential at 32 °C indicates a sharp change in the current through the sodium channels of the muscle membrane. Change in membrane fluidity or conformational change of the channel protein has previously been proposed as the underlying cause for the change in properties of other ionic channels[1–3]. It is also

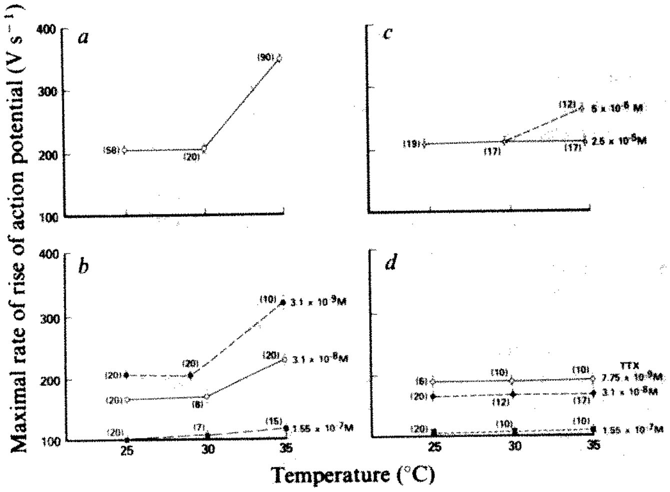

Fig. 2 Effect of imidazole and TTX on the maximal rate of rise of the action potential of the rat extensor digitorum longus at different temperatures. *a*, Control; *b*, effect of TTX. At a low TTX concentration (3.1×10^{-9} M) only the rate at 35 °C showed a significant reduction. At 3.1×10^{-8} M there was in addition a parallel shift of the rate between 25 and 30 °C. At 1.55×10^{-7} M, the difference in rate at different temperatures was completely eliminated. *c*, Effect of imidazole. Note that imidazole was effective in reducing the rate of rise only at 35 °C. The inhibition by imidazole was limited to the level as the rate of rise at low temperature. *d*, Effect of TTX after pretreatment with imidazole (10^{-4} M). Imidazole at 10^{-4} M abolished the difference in the rate of rise with respect to temperature. The effect of TTX in such conditions was to produce an inhibition that was not sensitive to temperature.

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*Present address: Department of Physiology, Monash University, Clayton, Victoria 3168, Australia.

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possible that there is a separate population of temperature-sensitive channels.

The finding that imidazole only exerts its action at temperatures above 32 °C and reduces the rate of rise to that of the lower temperatures is difficult to explain. Whether imidazole can prevent changes in membrane fluidity or protein conformation has yet to be determined. Alternatively, the results may be interpreted to indicate two components contributing to the maximal rate of rise of the action potential: a temperature-independent component and a temperature-dependent component selectively blocked by imidazole. The latter is also more sensitive to TTX. In such a case, the action of imidazole may be due to its molecular structure, so that it selectively blocks the sodium channels activated at higher temperatures. If this were the correct interpretation, these results then implicate the existence of two types of sodium channel that are pharmacologically distinguishable using imidazole. The possibility that thromboxanes or the adenylate cyclase system may be involved in the regulation of the sodium current may also have to be considered as imidazole is a potent inhibitor of thromboxane synthesis[7] as well as an activator of adenyl cyclase.

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Different components of black widow spider venom mediate transmitter release at vertebrate and lobster neuromuscular junctions

L. C. Fritz, M.-C. Tzeng* & A. Mauro

The Rockefeller University, New York, New York 10021

Crude black widow spider venom (BWSV) has profound physiological effects on several neuromuscular preparations, both vertebrate and invertebrate[1]. At frog and mouse neuromuscular junctions (NMJs), BWSV causes a massive increase in the frequency of miniature endplate potentials (m.e.p.ps)[2,3] followed by a reduction in m.e.p.p. frequency and depletion of synaptic vesicles[3–5]. Qualitatively similar physiological and morphological effects are also observed at lobster[6–8] and insect[9,10] NMJs after treatment with BWSV. Apparently, therefore, this venom can cause the release of several transmitters—acetylcholine at vertebrate NMJs and γ-aminobutyric acid (GABA) and glutamate at invertebrate NMJs[11]. BWSV has also been shown to cause the release of acetylcholine, noradrenaline and GABA from slices of mouse cerebral cortex[12,13]. α-Latrotoxin, a protein of molecular weight (MW) 130,000, had previously been shown to be responsible for the venom effects at vertebrate NMJs and in mouse brain slices[12–14]. That finding prompted the question of whether α-latrotoxin was also responsible for transmitter release in the lobster preparation. The present report demonstrates that although the electrophysicological effects of BWSV on lobster and frog NMJs are similar, they are caused by different components of the venom. The effects on the lobster are attributable to fraction E, which contains a major 65,000-MW protein and several lower molecular weight species. It has previously been shown that fraction E causes firing of the crayfish stretch receptor[14]. Some of these results have been published elsewhere[15].

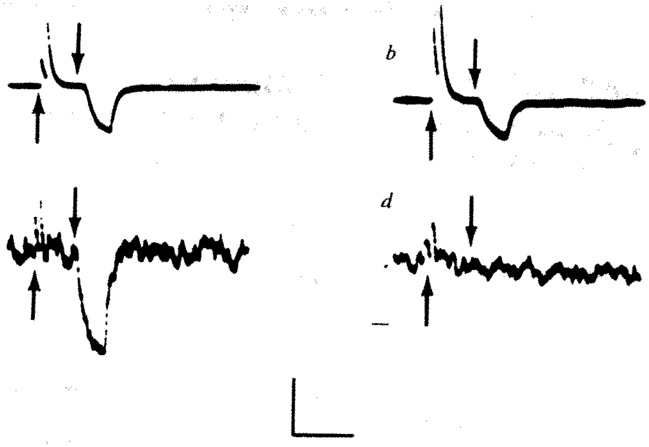

Fig. 1 Effect of fraction E on the lobster neuromuscular junction. In each trace, a pair of stimuli was applied to the excitatory axon (eliciting excitatory postsynaptic potentials) at the upward arrow; a train of stimuli (60 s^{-1}) was applied to the inhibitory axon (eliciting a summated inhibitory postsynaptic potential) at the downward arrow. *a*, Control; *b*, *c*, *d*, 17, 24, 28 min, respectively, after addition of 9 μg of fraction E. The excitatory potentials are potentiated in *b*, and the inhibitory potential is potentiated in *c*. In *d*, both the excitatory and inhibitory potentials are greatly reduced in amplitude. The ragged baseline in *c* and *d* is caused by a high frequency of spontaneous miniature potentials. Calibration: 2 mV, 1 s.

Venom glands were extracted from adult females of the black widow spider, *Latrodectus mactans tredecimguttatus*. Crude venom was prepared by homogenising two glands in 0.25 ml of lobster Ringer; venom was fractionated as previously described[14]. Intracellular recordings from the stretcher muscle of the lobster walking leg and from the frog cutaneous pectoris muscle were made using conventional techniques[16,17]. Superficial fibres from the lobster muscle were exposed by chipping away the overlying shell and removing the connective tissue layers. A small pool (~0.2 ml) was formed around the exposed fibres with Vaseline and toxins were added to the pool. Cutaneous pectoris muscles were pinned out in a chamber (~3 ml) to which toxins were added.

In the lobster, crude BWSV initially causes a potentiation of excitatory and inhibitory postsynaptic potentials, both of which are subsequently abolished, and a great increase in the frequency of miniature excitatory postsynaptic potentials[6]. Figure 1 demonstrates that these physiological effects can be elicited by application of 9 μg of fraction E. In contrast, Fig. 2 shows that at the frog NMJ, even after 2 h of exposure to 12 μg of fraction E, no increase in m.e.p.p. frequency is observed. However, subsequent treatment with 1 μg of α-latrotoxin rapidly causes a large increase in m.e.p.p. frequency (Fig. 2*d*). To test whether the apparent inactivity of fraction E on the frog NMJ in the above experiments was due to the large volume of fluid in the chamber (~3 ml), some additional experiments were carried out in which fraction E was applied to small (~0.2 ml) Vaseline-enclosed pools surrounding endplate regions. Even in these conditions, no rapid increase in m.e.p.p. frequency was observed, although after prolonged exposure (1–2 h) to a large dose (36 μg) of this fraction, high frequency bursts of m.e.p.ps were seen. This low level of activity is most probably attributable to slight contamination of fraction E by α-latrotoxin, as a similar level of activity is observed after prolonged exposure to low doses of α-latrotoxin, and such contamination can be demonstrated by SDS-polyacrylamide gel electrophoresis.

Although the lobster NMJ is sensitive to fraction E, Fig. 3 demonstrates that it is insensitive to α-latrotoxin. Following the addition of 2 μg of α-latrotoxin, no potentiation of evoked responses and no increase in the frequency of spontaneous miniature potentials were observed. Subsequent addition of 50 μl of crude BWSV, however, caused its usual effect, verifying that the preparation was still capable of responding to the crude venom.

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* Present address: Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan.

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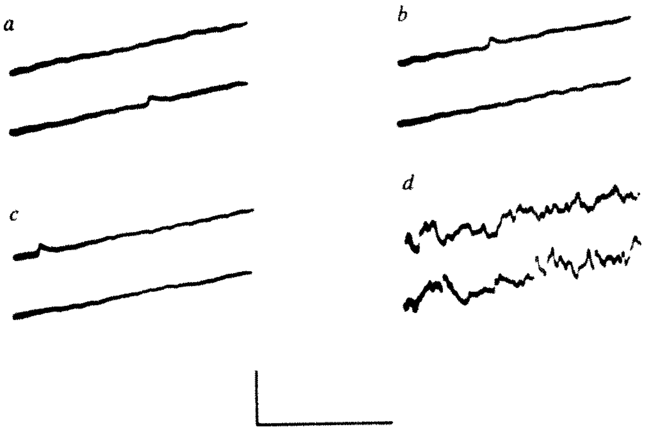

Fig. 2 The absence of an effect of fraction E on the frog neuromuscular junction. *a*, Control; *b* and *c*, 18 and 136 min, respectively, after addition of 12 μg fraction E to the bath. Individual m.e.p.ps can be seen in *a*, *b* and *c*. α-Latrotoxin, 1 μg, was added 168 min after fraction E addition. *d*, 14 min after the α-latrotoxin application. A high m.e.p.p. frequency is evident in *d*. Calibration: 2.5 mV, 50 ms.

These experiments show that fraction E can account for all the physiological effects of BWSV on the lobster NMJ, whereas α-latrotoxin is inactive. In contrast, α-latrotoxin causes the release of every transmitter so far examined in vertebrates[12–14]. The present experiments also confirm that fraction E does not cause the release of transmitter at the frog NMJ[14]. Fraction E has previously been shown to be ineffective in stimulating the release of GABA from mouse brain slices[13].

α-Latrotoxin binds with high affinity to some protein components in neuronal membranes[18], possibly binds also to gangliosides[19], and apparently acts, at least in part, by forming ionic channels[20,21]. It seems possible, then, that the inactivity of α-latrotoxin at the lobster NMJ reflects an absence of toxin receptors on the lobster nerve terminal and/or an inability of the toxin to become incorporated into the lobster nerve terminal membrane. Similarly, membrane components that interact with fraction E may be present on nerve terminals of lobster but not of frog. We cannot rule out the possibility, however, that α-latrotoxin and fraction E do not have equal access to nerve endings of lobster and frog. The calcium ionophore X-537A, on the other hand, seems to interact with both frog and lobster nerve terminals, as it is capable of increasing miniature potential frequency in both these preparations[22].

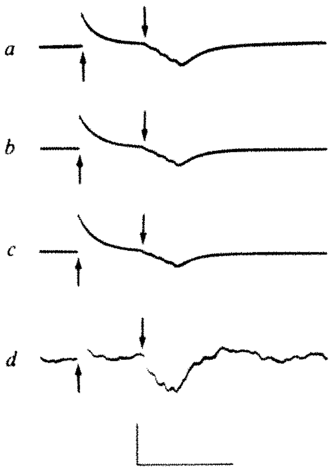

Fig. 3 The absence of an effect of α-latrotoxin on the lobster neuromuscular junction. In each trace, one stimulus was applied to the excitatory axon at the uppward arrow; a train of four stimuli ($10\ s^{-1}$) was applied to the inhibitory axon at the downward arrow. *a*, Control; *b*, and *c*, 10 and 20 min, respectively, after addition of 2 μg α-latrotoxin. 50 μl of crude BWSV was added 25 min after the α-latrotoxin. *d*, 10 min after the addition of the crude BWSV. The ragged baseline in *d* is due to a high frequency of miniature potentials induced by the venom. Calibration: 5 mV, 1 s.

BWSV causes transmitter release from insect NMJs[9,10] and the component which affects the cockroach NMJ has a MW of 125,000 (ref. 10). It seems likely that this component is a constituent of fraction C described by Frontali *et al.*[14], as this fraction is the only one to affect houseflies and cockroaches in their assay. Assuming this is true, the crude venom contains at least three separate protein components, each capable of causing transmitter release, but each demonstrating some degree of species specificity.

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Dopamine stimulates the degradation of gonadotropin releasing hormone by rat synaptosomes

D. Marcano de Cotte, C. E. L. De Menezes,
G. W. Bennett & J. A. Edwardson*

Department of Physiology, St George's Hospital Medical School,
Tooting, London SW17, UK

Immunohistochemical studies have shown the existence of synapses between nerve endings containing dopamine and gonadotropin releasing hormone (LH-RH) respectively in the hypothalamic median eminence[1]. It is generally accepted that this transmitter is involved in the regulation of gonadotropin secretion as a modulator of LH-RH release, although the evidence is conflicting and both inhibitory and excitatory actions of dopamine have been reported[2,3]. *In vitro* studies support the excitatory effect and low doses of dopamine have been shown to release LH-RH from incubated hypothalamic fragments[4,5] and synaptosomes[6]. This report describes studies with nerve endings isolated from the rat hypothalamus in which dopamine at higher concentrations than those used previously has been found to stimulate the degradation of LH-RH but not thyrotropin releasing hormone (TRH). This mechanism seems to be physiological in that it is calcium-dependent, requires the structural integrity of the nerve endings and fluctuates with the reproductive state of the animal.

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* To whom correspondence should be addressed at: Medical Research Council Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne, UK.

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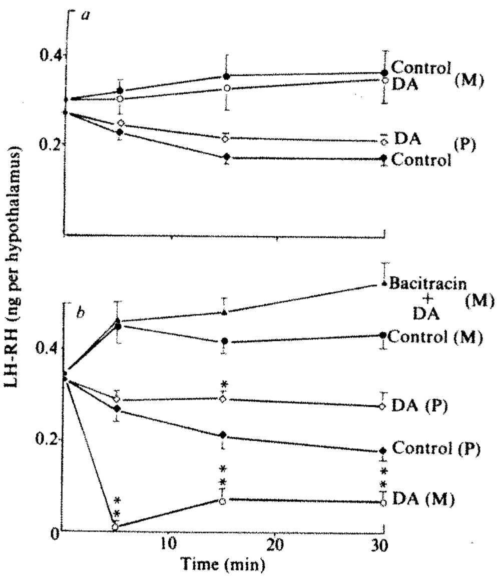

Effect of 10^{-4} M dopamine (DA) on the release of oreactive LH-RH from synaptosomes isolated from male and female (*b*) rat hypothalamus. Adult male and female Wistar rats (200–300 g) were housed in a temperature-controlled animal room with lights on from 6.00 to 18.00 h. The animals were stunned and decapitated. The entire hypothalamus was rapidly removed and synaptosomes were isolated and suspended in Krebs–Ringer bicarbonate medium containing glucose, at a tissue concentration equivalent to 1 hypothalamus per ml, using methods described previously[13]. The synaptosomes were incubated at 37 °C in capped plastic vials for a preincubation period of 15 min before the addition of dopamine, added in 20 µl of 1 mM ascorbic acid, to give a final concentration of 10^{-4} M. An equal volume of the ascorbic acid was added to control incubations. After incubation the synaptosomes were deposited by centrifugation (1,000*g* for 10 min) at room temperature and both the supernatant medium and synaptosomal pellets stored at −40 °C. Pellets were extracted with 0.5% Triton-X100 (ref. 14) and both LH-RH[15] and TRH[16] measured by immunoassay. Details of the specificity of the antibody used to measure LH-RH have been described elsewhere[17]. The antiserum does not recognise the peptide fragments produced after cleavage at the Gly^6-Leu^7 bond, the major mechanism for inactivation[10], but it does cross-react with LH-RH after removal of the COOH-terminal glycinamide, the other major point of endogenous peptidase activity[10]. The antiserum against TRH does not cross-react with pyroGlu-His-Pro-OH, pyroGlu-His or pyroGlu-Met-ProNH$_2$ and in studies on endogenous TRH-like material separated by HPLC using several different solvent systems, cross-reaction occurs only against a single fraction with properties identical to synthetic TRH. Dopamine at the concentrations used in the experiments did not interfere with the immunoassays. This aspect was rigorously tested. Small differences in the standard curve for LH-RH were obtained in the presence of 0.5% Triton and two assays were run systematically, one including Triton which was used to estimate the LH-RH content of the extracted pellets. Curves show levels of immunoreactive LH-RH present in the incubation medium (M), and Triton extracts of the synaptosomal pellets (P). In preparations from both sexes approximately two thirds of the total tissue content of LH-RH was released after 30 min control incubation, a finding which we have discussed elsewhere[18]. In *b* the levels of LH-RH released in the presence of bacitracin alone were similar to those obtained with bacitracin plus dopamine, and have not been shown for the sake of clarity. Values are expressed as mean ± s.e.m. in 9 to 31 determinations. The statistical significance of differences was determined with a two-tailed *t*-test. Different from control groups at: $*P<0.05$; $**P<0.001$.

Preliminary studies were carried out with hypothalamus from female rats at random stages of the oestrous cycle and from male animals. Figure 1 shows the basal release of immunoreactive LH-RH from synaptosomes, during a 30-min incubation period. Levels of LH-RH in the incubation medium were relatively high to begin with, because of release during the preincubation period, and increased only slightly during the next 30 min. The content of LH-RH in the synaptosomal pellet obtained after centrifugation showed a decline with time, indicating depletion of a releasable pool of the neuropeptide. In the presence of dopamine (10^{-4} M) there was a significant reduction in the level of LH-RH in the medium from female hypothalamic synaptosomes. This was maximal after 5 min, when almost all of the LH-RH released during the period of preincubation had disappeared. The disappearance of immunoreactive LH-RH from the medium after dopamine treatment is unlikely to be due to re-uptake or inhibition of release as the LH-RH content of the pellet was unchanged at this time, although the possibility of re-uptake coupled with intracellular degradation cannot be excluded. After 15 and 30 min of incubation the concentration of LH-RH in the synaptosomal pellets in the presence of dopamine was raised, but the preparation still showed a significant net loss of LH-RH ($P<0.005$). Furthermore, the action of dopamine was blocked in the presence of bacitracin (5×10^{-4} M), an antibiotic peptide which competitively inhibits the degradation of LH-RH by peptidases[7]. In contrast to this effect of dopamine on LH-RH in synaptosomes isolated from female hypothalamus, dopamine did not reduce the LH-RH level in the medium from synaptosomes isolated from the male rat hypothalamus.

Subsequent experiments were carried out with synaptosomes from female rats at defined stages of the oestrous cycle and incubations with dopamine were carried out for 5-min periods. Figure 2 shows that although a significant effect of dopamine was obtained at every stage of the reproductive cycle, the degradation of LH-RH was most marked in the pro-oestrous preparation. Although the values are not shown, the concentrations of LH-RH in the synaptosomal pellets did not vary with different stages of the oestrous cycle and were not affected by the addition of dopamine. Figure 2 also shows that in the same preparations there was a significant increase in the release of immunoreactive TRH in the presence of dopamine, an effect which we have also observed with nerve endings from sheep hypothalamus[5]. Dopamine did not alter the synaptosomal content of TRH.

The effect of dopamine on the degradation of exogenous LH-RH was also studied using synaptosomes isolated from

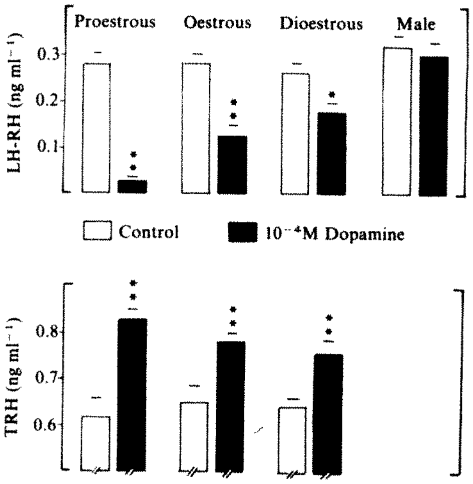

Fig. 2 Levels of immunoreactive LH-RH and TRH in the supernatant medium from synaptosomes isolated from female rat hypothalamus at different stages of the oestrous cycle. Daily vaginal smears were taken in the morning and only rats showing at least 2 or 3 successive regular 4-d cycles were used. Experimental conditions were identical to those described for Fig. 1. Values show levels of peptide present after 15 min preincubation followed by 5 min incubation in the presence of dopamine or ascorbic acid control vehicle, and represent the mean ± s.e.m. in 13 to 18 determinations. The statistical significance of differences was determined by the two-tailed *t*-test. Different from control groups at: $*P<0.025$; $**P<0.001$.

female rats at random stages of the oestrous cycle. After preincubation, 10 ng of synthetic LH-RH was added, an amount almost 30-fold greater than endogenous levels released by the synaptosomes. Dopamine (10^{-4} M) stimulated the disappearance of exogenous LH-RH from the medium and bacitracin completely inhibited this effect (Fig. 3). Exogenous LH-RH was also added to suspensions of cerebral cortex synaptosomes incubated at a tissue concentration equivalent to 1/3 cortex per ml. Substantial degradation of LH-RH occurred in these conditions but dopamine did not have any significant effect on the rate of breakdown. The effect of removal of free calcium on dopamine-induced disposal of both endogenous and exogenous LH-RH by hypothalamic synaptosomes was also studied. When calcium was omitted from the Krebs–Ringer bicarbonate buffer, the degradation of LH-RH in the presence of dopamine was not observed (Fig. 3).

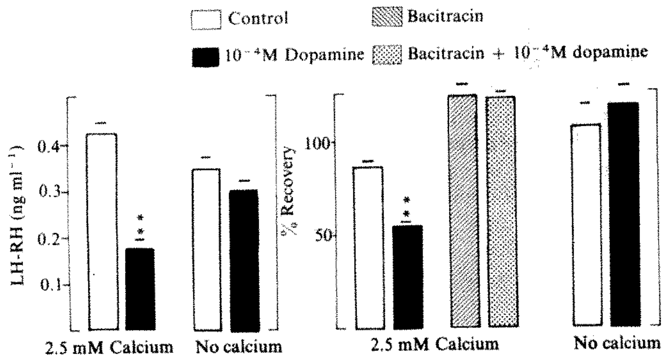

Fig. 3 Effect of calcium on the dopamine-induced degradation of endogenous (left) and exogenous (right) LH-RH. Percentage of recovery was calculated with each experiment from controls consisting of the various media incubated with 10 ng synthetic LH-RH. Values greater than 100% obtained when bacitracin was present or calcium was omitted, are presumably due to the decreased breakdown of endogenously released LH-RH in the medium. The synaptosomes were incubated for 30 min in the presence of LH-RH and other agents. The LH-RH content of the synaptosomal pellets was not significantly altered in the presence of dopamine. Values are expressed as mean±s.e.m. in 9 to 32 determinations. The statistical significance of differences was determined by the two-tailed *t*-test. ** Different from control groups at $P < 0.001$.

The concentration of dopamine (10^{-4} M) required to stimulate the degradation of LH-RH by synaptosomes is relatively high. However, recently it has been reported that concentrations of dopamine in plasma from hypophysial portal blood are between 10^{-8} and 10^{-7} M (ref. 8). If this measurement is correct it is likely that much higher concentrations of dopamine occur in synaptic clefts or extracellular space surrounding dopaminergic nerve terminals. The concentration of acetylcholine in the neuro-muscular junction exceeds 10^{-4} M during stimulation[9] and there is no reason to suppose that such levels of neurotransmitters are not found in synaptic clefts elsewhere.

When synaptosomes were ruptured by sonication, dopamine did not stimulate the degradation of 10 ng synthetic LH-RH added to the preparations, indicating that the mechanism of this effect does not involve a direct action on the enzyme. Also, the supernatant medium from incubated synaptosomes did not significantly degrade LH-RH. Together, these results suggest an extremely complex mechanism which requires the structural integrity of the nerve ending and in which dopamine, ovarian steroids and calcium are all involved. The existence of such a system for the degradation of LH-RH may partly explain the apparent lack of correlation between the distribution of LH-RH and the peptidases which degrade it. Previous studies[10] have reported that less than 5% of LH-RH degrading activity is associated with the synaptosomal fraction, which contains most of the peptide. However, the membrane or cytosol preparations used in such studies lack the cellular organisation that the dopamine-sensitive, synaptosomal mechanism requires.

That dopamine-induced breakdown of LH-RH observed in this study may reflect a physiological mechanism is suggested by: (1) the differential effect of dopamine in rats at different stages of the oestrous cycle; (2) the lack of effect of dopamine on LH-RH degradation on male hypothalamic synaptosomes; (3) the inability of dopamine to enhance LH-RH disposal in a calcium-free medium; (4) the absence of any action on TRH breakdown; and (5) the finding that dopamine did not stimulate the disposal of exogenous LH-RH by synaptosomes from cerebral cortex. We suggest, therefore, that in addition to controlling the release of hypothalamic LH-RH dopamine may acutely regulate the disposal of this hormone by controlling peptidase activity at the nerve terminal. Such a mechanism could be important with relation to both the endocrine functions of LH-RH in control of gonadotropin secretion and its reported actions on neuronal activity[11] and sexual behaviour[12]. It could also have more general significance for the functional relationships between aminergic and peptidergic neurones in the central nervous system.

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Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells

K. Kumakura, F. Karoum*, A. Guidotti & E. Costa

Laboratory of Preclinical Pharmacology and * Laboratory of Clinical Psychopharmacology, National Institute of Mental Health, Saint Elizabeths Hospital, Washington, DC 20032

Morphological, physiological and pharmacological evidence indicates that opioid peptides, which in the brain are located intraneurally[1], may function as neurotransmitters. Similar evidence is not yet available for the opioid peptides that are stored in chromaffin cells of adrenal medulla[2–5] and in axon terminals located in adrenal medulla and sympathetic ganglia[3–5]. The present report contributes evidence suggesting that the opioid peptides which are stored in the axon terminals of the splanchnic nerves located in adrenal medulla[4] may function as

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neuromodulators of the acetylcholine receptors located on chromaffin cells that are involved in catecholamine release. We support this functional role of the opioid peptides by showing that primary cultures of chromaffin cells of bovine medulla contain opiate receptors. When these receptors are occupied by specific agonists, the number of nicotinic receptors and the amount of catecholamine released by maximal doses of nicotine are reduced. Thus, like in other neuronal systems[6–8] also in adrenal medulla, the action of opioid peptides is inhibitory. The specificity of this action is in part supported by the inability of opiate receptor agonists to reduce the Ca^{2+}-dependent release of catecholamines elicited by K^+ ions.

A highly purified primary culture of chromaffin cells was obtained from bovine adrenal medullae by a modification of the method described by Waymire *et al.*[9]. Approximately 10^8 chromaffin cells were obtained from a single bovine adrenal (for further details see Table 1 legend). These chromaffin cells can be kept in culture, without significant morphological or biochemical changes, for at least 2 weeks[10]. The chromaffin cells in culture retain the ability to release endogenous catecholamines in response to nicotinic receptor stimulation[10,11]. The nicotine-induced release of catecholamines is Ca^{2+} dependent[10].

Nicotinic receptor antagonists such as hexamethonium abolish this response[10,11], which is unaffected by pretreatment with blockers of muscarinic receptors[10]. This finding agrees with reports that in these primary cultures of chromaffin cells the number of high-affinity binding sites for ligands (^{3}H-quinuclidinyl benzilate) of muscarinic receptor sites[10] is very small.

The experiments reported here were carried out in cells maintained in culture for 1 week. To study catecholamine release, 10^6 cells were washed once with phosphate-buffered saline and suspended in 1 ml of Krebs–Ringer bicarbonate–glucose buffer (*p*H 7.2–7.4) and 0.5% bovine serum albumin at 37 °C. The content of catecholamines and their metabolites in the cells was measured by mass-fragmentography as previously reported[12,13], with minor modifications. The cells were homogenised in 100 µl of 1 M HCl solution containing 25 ng each of deuterated adrenaline, noradrenaline and dopamine. These internal deuterated amines were used to quantify the endogenous amines by direct comparison. Adrenaline (15 ± 0.8 nmol per 10^6 cells; $n = 3$) represents approximately 75%, noradrenaline (3.4 ± 0.2 nmol per 10^6 cells; $n = 3$) 17% and dopamine (1.3 ± 0.06 nmol per 10^6 cells) 7%, of the total catecholamine content. Only trace amounts of dihydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were detected. A rapid spontaneous release of catecholamines was observed during the first 5 min of incubation in Krebs buffer. During this period approximately 1–2% (0.2–0.4 nmol per 10^6 cells) of the total catecholamine stores was released. The spontaneous release of catecholamines decreased to 0.5% of the total cell content in the following 5-min period. During a 3-min exposure to nicotine (10^{-5} M) or a high potassium concentration

Table 1 Effect of morphine and other opiate receptor agonists on the release of endogenous catecholamines from cultured adrenal chromaffin cells

Secretory stimulus	Opiate receptor agonist		Catecholamine secretion (nmol per 10^6 cells)	%
Nicotine 10^{-5} M	None		2.0 ± 0.15	100
	Morphine	10^{-7} M	1.8 ± 0.15	89
		10^{-6} M	1.5 ± 0.06*	75
		10^{-5} M	1.3 ± 0.08*	65
		10^{-4} M	1.1 ± 0.09*	56
	Morphine	10^{-5} M + NTL 10^{-5} M	2.2 ± 0.20†	109
		10^{-4} M + NTL 10^{-4} M	1.5 ± 0.10†	75
Nicotine 10^{-5} M	None		2.3 ± 0.20	100
	β-Endorphin	10^{-9} M	1.9 ± 0.20	81
		10^{-8} M	1.7 ± 0.09*	76
		10^{-7} M	1.6 ± 0.10*	69
		10^{-6} M	1.1 ± 0.12*	49
		10^{-7} M + NTL 10^{-7} M	2.2 ± 0.19†	94
Nicotine 10^{-5} M	None		2.1 ± 0.15	100
	Met-enkephalin	10^{-5} M	2.4 ± 0.29	116
		10^{-4} M	1.5 ± 0.12*	70
		10^{-3} M	0.8 ± 0.10*	38
	FK33-824	10^{-7} M	0.7 ± 0.07*	35
		10^{-6} M	0.6 ± 0.07*	28
KCl 56 mM	None		2.4 ± 0.23	109
	Morphine 10^{-4} M		2.4 ± 0.30	108

Adrenal chromaffin cells were isolated from fresh bovine adrenal glands as described by Waymire *et al.*[9]. Briefly, the procedure consists of: dissection of the adrenal medulla from the cortex, digestion of the interstitial tissue of adrenal medulla by retrograde perfusion with 0.25% collagenase, differential centrifugation and filtration, and differential plating to remove non-chromaffin cells. The chromaffin cells were cultured in F-12-10% fetal calf serum medium. The purity of the chromaffin cell preparation was established microscopically with the specific method for catecholamine fluorescence and Giemsa staining[23]. The contaminant cells were always less than 5%. The chromaffin cells were able to maintain a constant level of catecholamines (20 nmol per 10^6 cells) and related enzymes for 1–2 weeks[10,11]. Large numbers of cells could be purified with this procedure. Approximately 10^8 cells were obtained from a single bovine adrenal medulla. For the release studies approximately 10^6 cells per sample were used. The release of endogenous catecholamines from cultured chromaffin cells was measured by incubating the cell suspension at 37 °C for 2.5 min in Krebs–Ringer bicarbonate–glucose buffer (*p*H 7.2–7.4) and 0.5% bovine serum albumin with 2.2 mM $CaCl_2$. The cell suspension was filtered through a Millipore filter after the incubation. The released catecholamines (adrenaline + noradrenaline) were extracted from the filtrate with 0.4 M perchloric acid and assayed fluorimetrically after Al_2O_3 adsorption[14]. 'Catecholamine secretion' represents the amount of catecholamines released (in 2.5 min) by nicotine or K^+ ions after subtracting the baseline release of catecholamines. The baseline release accounts for 20–30% of the total catecholamines present in the medium after nicotine or KCl stimulation. Each value is the mean ± s.e. of two or three separate experiments. In each experiment the effect of drugs (added 15 min before 10^{-5} M nicotine) was tested in groups of three separate samples. There was no significant release of the endogenous catecholamines by the opiate substances themselves at the doses used. Regression analysis of the dose–response curve for different opiate receptor agonists give the following coefficient of correlation (r^2) values: morphine, 0.99 ($P < 0.01$); β-endorphin, 0.88 ($P < 0.01$).

* Statistically significant ($P < 0.05$) when the treated groups are compared with the control group, using the *t*-test pair analysis.

† Statistically significant ($P < 0.05$) when morphine-treated groups are compared with the corresponding groups treated with morphine + naltrexone (NTL).

(56 mM), there was a large linear increase in the rate of catecholamine release which was 10 to 15 times greater than the spontaneous release rate.

During the 3-min stimulation period, 10–15% of the total catecholamine cell content was released. After this very high release there was a 3-min period of much slower release during continuous nicotine or potassium stimulation. We measured the relative proportion of adrenaline, noradrenaline and dopamine released before and after nicotine stimulation with or without opiate treatment by mass-fragmentography and found that the medium contained, in all the conditions studied, approximately 55% adrenaline, 45% noradrenaline and 1% dopamine. Only trace amounts of DOPAC and HVA were present. The catecholamine content remaining in the cells after the release experiments and the relative proportions of catecholamines present were mostly the same as those measured in control preparations. Due to the large amounts of catecholamines available in each sample (approximately 2–4 nmol in the supernatant and 20 nmol in the cells) and based on the mass-fragmentographic data indicating that practically only adrenaline and noradrenaline were released, we found it convenient in the experiments reported in Table 1 and Fig. 1 to assay the catecholamines (adrenaline + noradrenaline) with the spectrofluorimetric method[14].

Table 1 shows that morphine reduces the release of catecholamines elicited by the addition of a maximally active dose (10^{-5} M) of nicotine. This inhibitory action of morphine is dose related and is abolished by the presence of naltrexone, a potent opiate receptor antagonist. The peptides β-endorphin and FK33-824 (a stable analogue of enkephalin[15]; Sandoz) also produced a statistically significant dose-related inhibition of the nicotine-stimulated catecholamine release (Table 1). These peptides are several orders of magnitude more potent than morphine or Met-enkephalin (Table 1). The action of opiate receptor agonists cannot be due to an increase in the degradation rate of catecholamines because when ^{3}H-noradrenaline and ^{3}H-adrenaline were incubated for 3 min with the incubation media obtained from cells treated with opiate receptor agonists in the presence or absence of 10^{-5} M nicotine, no loss of ^{3}H-labelled catecholamines was measured. In addition, the effect of opiates cannot be due to changes in the cell catecholamine content because the cell content failed to change with the addition of opiate receptor agonists (20 ± 1.8 nmol per 10^6 cells in controls; 19 ± 1.5 nmol after 10^{-4} M morphine; 21 ± 2.0 after 10^{-7} M β-endorphin). These experiments suggest that opiate receptor agonists inhibit the release of catecholamines elicited by the stimulation of nicotinic receptors and that this action requires the availability of those receptor sites which are blocked by naltrexone. We then studied whether crude P_2 preparations of membranes obtained from chromaffin cell cultures contain specific sites for high-affinity binding of ^{3}H-Met-enkephalinamide and ^{3}H-naloxone (Fig. 1). The K_D values calculated from Scatchard plots of the saturation curves are 0.9 nM for Met-enkephalinamide and 3.2 nM for naloxone. These values are close to those calculated for opiate receptors present in brain membrane preparations[16].

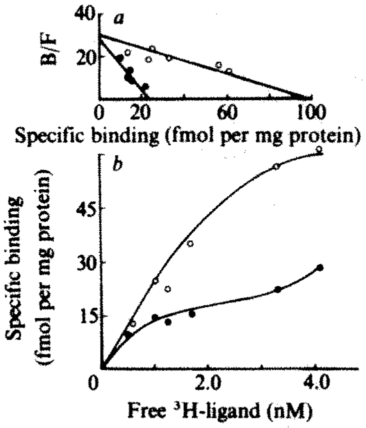

Fig. 1 Scatchard plots (*a*) and saturation curves (*b*) of ^{3}H-(D-Ala^2-L-Met^5)enkephalinamide (●) (37.7 Ci mmol^{-1}), and ^{3}H-naloxone (○) (14.6 Ci mmol^{-1}, both NEN) binding to cell membranes. The cells were homogenised in 50 mM Tris-HCl (*p*H 7.0) and the homogenate was centrifuged at 12,000*g* for 15 min. The pellet was resuspended in 50 mM Tris-HCl (*p*H 7.0) and preincubated at 25 °C for 1 h[16]. After the second centrifugation at 12,000*g* for 15 min, the pellet was resuspended in 50 mM Tris-HCl (*p*H 7.0). Specific binding was calculated by subtracting from the binding which occurred by incubating the pellet (approximately 100 μg protein) at 25 °C for 1 h with ^{3}H-labelled ligand, the binding which occurred by incubating with ^{3}H-labelled ligand plus 10^{-3} M morphine in a total volume of 600 μl. After incubation the reaction was terminated by centrifugation at 12,000*g* for 15 min. The supernatant was discarded and the pellet was rapidly rinsed with 1 ml of ice-cold 50 mM Tris-HCl (*p*H 7.0). The pellet was dissolved in 50 μl of NCS (Amersham tissue solubiliser) before counting the radioactivity with a liquid-scintillation counter. Proteins were determined with the method of Lowry[24]. B/F = [bound ^{3}H-ligand (fmol per mg protein)]/[free ^{3}H-ligand (nM)].

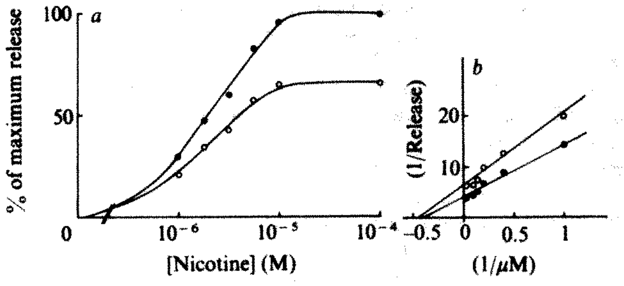

Fig. 2 Effect of morphine on the nicotine-induced release of endogenous catecholamines from cultured adrenal chromaffin cells. *a*, Release of endogenous catecholamines by various concentrations of nicotine in the absence (●) and presence (○) of 10^{-4} M morphine. *b*, Double-reciprocal plot of the data in *a*. The release of the endogenous catecholamines from cultured adrenal chromaffin cells was measured as described in Table 1. Morphine was added to the incubation medium 15 min before nicotine. Each point is the mean of two separate experiments in which triplicate samples were assayed. The error was less than 10% of the mean.

The release of catecholamines elicited by the stimulation of nicotinic receptors is inhibited by opiate receptor agonists (see Table 1) and by hexamethonium[11], but the mechanisms involved are different. Opiate receptor agonists act non-competitively (Fig. 2) whereas hexamethonium acts competitively[11,17]. Figure 2*a* shows that the nicotine-induced catecholamine release is decreased by about 45% in cells preincubated with 10^{-4} M morphine (Fig. 2*a*). The double-reciprocal plot (Fig. 2*b*) of the dose–response curve indicates that the extent of the maximal response decreases, but that the concentration of nicotinic agonist which gives half-maximal response fails to change.

Morphine (10^{-4} M) does not inhibit the release of catecholamines induced by the depolarisation of the cells with KCl (Table 1). Thus, it seems that in the chromaffin cell membranes, the stimulation of opiate receptor changes the function of nicotinic receptors by a specific mechanism. To characterise this mechanism further, we have investigated whether the stimulation of opiate receptors located in the chromaffin cell membranes can influence the number of available nicotinic receptors. In these studies, ^{125}I-α-bungarotoxin was used as the ligand for nicotinic receptors[18,19]. This ligand in a concentration of 10^{-8} M causes a 50% reduction of the release of catecholamine induced by 10^{-5} M nicotine. When morphine

(10^{-4} M) was added to the primary cultures of chromaffin cells it reduced the number of ^{125}I-α-bungarotoxin binding sites by approximately 80%. In untreated cells, the B_{max} for α-bungarotoxin binding was 3.1 fmol per mg protein; this value decreased to 0.65 in cells treated with morphine (10^{-4} M). The decrease in the B_{max} was not associated with a significant change in the affinity constant; the K_D values were 2.8 and 3.1 nM in normal and morphine-treated chromaffin cells, respectively. Similar results were obtained when the chromaffin cells were treated with FK33-824.

In conclusion, opiate receptors are present in the membrane of adrenal chromaffin cells. The activation of these receptors causes a non-competitive inhibition of catecholamine release elicited by the stimulation of nicotinic receptors. Our data indicate that β-endorphin, FK33-824 and Met-enkephalin reduce the action of nicotine by decreasing the number of available nicotinic receptors. This action is probably indirect and may involve an allosteric modulation of the recognition sites for ligands of nicotinic receptors. Based on these data, it is tempting to speculate that when the Met-enkephalin-like material present in terminals of splanchnic nerves[3-5] is released by nerve impulses, it modulates the release of catecholamines induced by nicotinic receptor stimulation elicited by the concomitant release of acetylcholine. A recept report[20] indicates that substance P inhibits nicotinic activation of the chromaffin cells. However, the physiological significance of the latter experiment is difficult to establish because evidence was not provided for the presence of substance P or a substance P-like material in splanchnic nerve terminals. The presence of opiate receptors on chromaffin cell membranes may also suggest a role for opiate peptides released into the circulation from the pituitary[21] or the adrenal cells[1,22]. Although these cells respond to several opiate peptides, including β-endorphin, this sensitivity is very low relative to their actual concentration in blood.

The precise mechanisms whereby the endogenous ligands of opiate receptors modulate the number of functional nicotinic receptor sites present in chromaffin cell membranes is unclear. It seems, however, that in chromaffin cells the opiate peptides that probably co-exist with acetylcholine in splanchnic nerves can disable the function of nicotinic receptors. Hence, it may be interesting to consider the possibility that 'co-transmitters' function as 'enablers' of the receptor function for the primary transmitter.

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Amino acids evoke short-latency membrane conductance increase in pancreatic acinar cells

N. Iwatsuki & O. H. Petersen

Department of Physiology, The University, Dundee, UK

Sodium-gradient-driven amino acid transport is regarded as an important mechanism for cellular uptake in general as well as in pancreatic acini[1-6]. Electrophysiological experiments in kidney tubules and intestine have revealed that amino acids can cause sodium-dependent membrane depolarisation[7-11]. The mechanism of this depolarisation is unknown[10] as it is uncertain whether the depolarisation is accompanied by a membrane conductance increase[9,11]. The pancreatic acinar tissue would seem to be an ideal system for investigating the electrophysiological mechanism of action of amino acids because (1) the pancreas is the most active amino acid-accumulating tissue[12], (2) the basic electrophysiology of the pancreatic acinar cells is well characterised[13] and (3) a direct comparison can be made in the same cells between the electrical actions of the pancreatic secretagogues and the amino acids. We now show that L-alanine evokes a stereospecific membrane depolarisation accompanied by an increase in membrane conductance and that this membrane effect has a much shorter latency than the secretagogue response. The null (equilibrium) potential for the amino acid-evoked potential change corresponds to the sodium equilibrium potential.

Two separate KCl-filled microelectrodes were inserted into neighbouring cells in the same acinus of mouse pancreas (under visual control using a Zeiss (Jena) phase contrast microscope (×400) with long working distance) *in vitro* as previously described[14,15]. Up to two extracellular micropipettes were used for local application of acetylcholine (ACh), L- or D-isomers of alanine, L-proline, L-valine or L-glutamate. The substances were ejected from the pipettes by ionophoresis using a similar procedure to that used for local application of peptide secretagogues[16]. The composition of the physiological saline solution, prewarmed to 37 °C, flowing through the bath, was (mM): NaCl, 139; KCl, 4.7; $CaCl_2$, 2.56; $MgCl_2$, 1.13; D-glucose, 2.8; Tris, 3. *p*H was adjusted to 7.4 with HCl and the solution was gassed with 100% O_2.

Figure 1*a* shows membrane depolarisations evoked by microionophoretic application of L- and D-alanine. Although the same amino acid concentrations, stimulus parameters and relative positions of the micropipettes were used, the effect of D-alanine was small and inconspicuous compared with that of L-alanine. This was consistently found in 10 different experiments. The L-alanine-induced depolarisation resembled the depolarisation induced by secretagogues such as ACh, cholecystokinin-like peptides and bombesin-like peptides[13-16]. However, a close examination of the time course of the potential changes (Fig. 1*b*) revealed that the L-alanine-evoked membrane effect had a much shorter latency than the ACh-induced depolarisation. The minimum latency for muscarinic receptor activation of pancreatic acinar[17] as well as other cells[18] is about 0.3–0.5 s. Similar values have been found with peptide secretagogue-evoked effects[16]. In contrast, the latency for the action of L-alanine was <50 ms (the latency may well have been much shorter, but the artefact caused by the onset of the ejecting ionophoretic current unfortunately obscured the initial 50 ms of the potential recording). Also, in spite of the shorter delay of the amino acid effect (Fig. 1*b*) the rise-time was much longer than for ACh. These short-latency responses to L-alanine stimulation were observed in all 14 experiments of this kind carried out. The short-latency responses to L-alanine application show that there is no substantial diffusional barrier to close ionophoretic drug

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application and that therefore the relatively long latency for the action of ACh is genuine. The long latencies for secretagogue actions in the pancreas may be related to the time taken for an intracellular messenger, calcium, to reach a sufficient concentration to initiate the response[19]. Although the effect of ACh was blocked by atropine (1 μM), this had no effect on the L-alanine-evoked depolarisations (three experiments). Other amino acids were also tested, and L-valine (Fig. 2), L-proline, L-leucine and L-serine had effects similar to that of L-alanine. The latencies of the effects of L-valine and L-proline were determined in five experiments. In all these cases, short latencies of the same order of magnitude as found for L-alanine were observed. L-glutamate, however, had no effect on the bioelectrical properties in the five experiments in which it was tested.

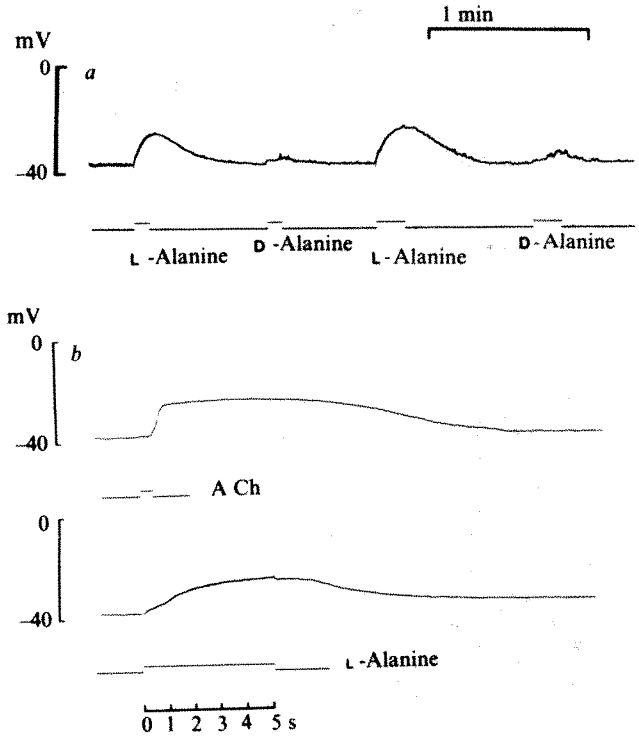

Fig. 1 *a*, Effects of ionophoretic L-alanine and D-alanine applications on the membrane potential in the same acinar cell (atropine (10^{-6} M) present). Micropipettes were filled with either 1 M L- or D-alanine (Sigma) dissolved in 0.9% NaCl (*p*H 4.0). The marker signals in the bottom trace indicate periods of L- or D-alanine ejection near to the surface of the impaled acinar cell, using positive ejecting currents of 100 nA and 5 s (short marker signals) or 10 s (longer marker signals) duration. No retaining current was used. *b*, Measurement of latency of responses to ionophoretic application of acetylcholine (ACh) and L-alanine from the same pancreatic acinar cell. Two extracellular micropipettes, filled with 2 M AChCl and 1 M L-alanine, respectively, were placed very close to the acinus impaled by the KCl-filled recording electrode. Upper trace: ACh stimulation was applied by a positive current of 80 nA (retaining current 20 nA) for 500 ms. Lower trace: L-alanine ionophoresis was applied by a positive current of 800 nA (no retaining current) for 5 s. The latency was taken as the interval between the start of the stimulus artefact and the take-off point arising from the depolarisation of the impaled acinar cell. A latency of 300 ms was found in the upper trace. The lower trace (L-alanine) shows the beginning of cell depolarisation less than 50 ms after the start of L-alanine ejection. The two traces are excerpts from a single continuous membrane potential recording from one cell.

We then tested the effect of superfusing the pancreatic acinar tissue with solutions containing known concentrations of L-alanine. At 0.1 mM, L-alanine caused a depolarisation of 3.3 mV ± 0.3 (s.e.)($n=7$), a 1-mM concentration depolarised the acinar membrane by 7.3 mV ± 0.2 ($n=6$), and at 10 mM, L-alanine evoked a reduction in membrane potential of 17.6 mV ± 1.0 ($n=7$). The effect of 20 mM L-alanine was not significantly different from that obtained at 10 mM (18.5 mV ± 0.5 ($n=5$)).

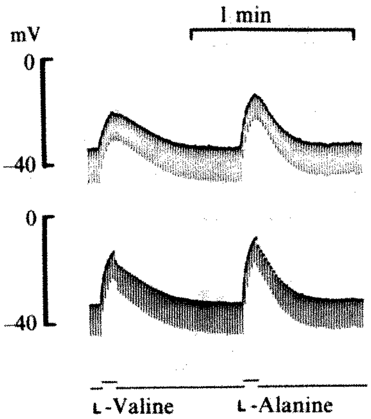

Fig. 2 Effects of microionophoretic application of L-valine and L-alanine on membrane potential and resistance in two electrically coupled neighbouring acinar cells. Current pulses (1 nA, 100 ms duration) were injected through the microelectrode recording the membrane potential represented by the lower of the two potential traces. Current pulses (500 nA and 5 s duration) were passed through the two extracellular micropipettes filled with 0.5 M L-valine or 1 M L-alanine, respectively, at marker signals in the bottom trace. The sudden small potential change corresponding to the end of ejecting current pulses seen in the lower potential trace represents the current artefact. (It is not seen in the upper trace, because that membrane potential was recorded using a differential amplifier.) Resting input resistance was 12 MΩ in this case. During the ionophoretic applications of L-valine and L-alanine the resistance was reduced to 10 and 8.5 MΩ, respectively.

The depolarisation evoked by L-alanine or L-valine was accompanied by a reduction in membrane resistance (Fig. 2). This was a consistent finding in the several hundred experiments carried out with L-alanine and the eight experiments with L-valine. Dose–response curves for L-alanine-evoked depolarisation and resistance reduction followed each other closely. The maximal reductions in input resistance obtained were to about 50% of the pre-stimulation value.

Replacing all the superfusion fluid Na by Tris (16 experiments) or Li (6 experiments) in all cases resulted in an immediate reduction in the amplitude of the L-alanine-evoked depolarisation. This effect was immediately reversible on re-admission of Na to the bath.

Figure 3 shows an attempt to determine the null (reversal) potential for the L-alanine-evoked potential change. In all seven experiments of the kind shown in Fig. 3, an approximately linear relationship between membrane potential and amplitude of L-alanine-evoked depolarisation was observed. An actual reversal of the potential change could not, however, be obtained because it proved impossible to attain membrane potentials

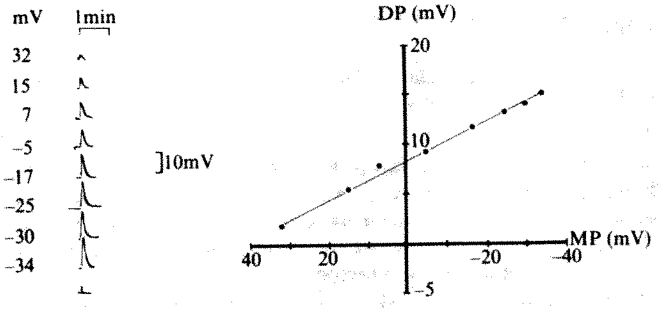

Fig. 3 To the left is shown the effect of L-alanine ionophoresis (1,000 nA ejecting 2 s duration) on the membrane potential of an acinar cell at different levels of resting potential (figures written to the left of individual records) in the same cell. Higher and lower resting potentials were obtained by passing direct hyper- or depolarising current, respectively, through a microelectrode inserted into an electrically coupled neighbouring cell. The L-alanine-evoked potential changes, plotted as a function of resting potential, are shown to the right. DP, depolarisation; MP, membrane potential.

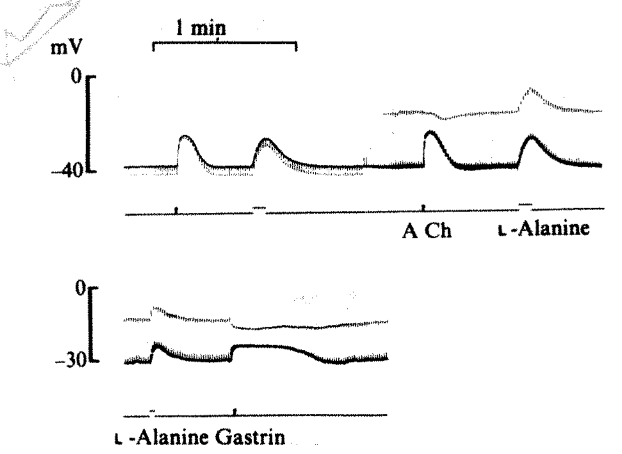

Fig. 4 Comparison of the membrane effects of L-alanine with ACh or gastrin in the same cells. In the upper trace ACh and L-alanine applied from two separate micropipettes by ionophoresis (80 nA, 0.5 s and 800 nA, 5 s, respectively) evoked depolarisations of similar size. During the first ACh and L-alanine stimulation, small hyperpolarising current pulses were repetitively injected through a microelectrode inserted into a neighbouring cell. Thereafter, the polarity of the injected current pulses was switched and the intensity increased (to 7 nA, 100 ms). In this way, the action of ACh and L-alanine could be simultaneously assessed at the spontaneous resting potential (heavy trace) (about −40 mV) and the artificially lowered potential (interrupted trace) (about −14 mV). The reduction in resting potential from −40 to −14 mV just reversed the polarity of the ACh-evoked membrane change, whereas the L-alanine-evoked depolarisation was only slightly reduced. The lower trace shows a similar comparison between L-alanine and gastrin actions in another cell. Gastrin was added directly (3.5 nmol) to the bath. At −30 mV, both substances evoked depolarisation. At −13 mV, the gastrin response was reversed but the L-alanine response was only slightly reduced in amplitude.

beyond +40 mV by direct current injection. Extrapolation of the linear curve in Fig. 3 to the intersection with the membrane potential axis (x-axis) revealed a null (reversal) potential of +40 mV. Values ranging from +20 to +60 mV were found in the other six experiments. These values are vastly different from the reversal potential of −10 to −20 mV repeatedly observed for the potential changes evoked by several different pancreatic secretagogues[14,16]. The null potential for the secretagogue action of about −15 mV has previously been shown to be due to increases in both chloride and sodium conductance, with some contribution also from potassium[14]. Figure 4 shows that current-evoked membrane depolarisations just sufficient to cause reversal of the effects of ACh or gastrin fail, in the same cells, to reverse the L-alanine-evoked depolarisation. In mouse pancreatic segments superfused *in vitro*, the intracellular sodium concentration, $[\mathrm{Na}]_i$, is about 42 mM (ref. 20), therefore, the Na equilibrium potential (E_{Na}) at 37 °C is about +33 mV. The null (reversal) potential for the action of L-alanine seems to be very close to this value. Null potentials (extrapolated) of +20 to +40 mV were estimated for L-valine and L-proline in four experiments similar in nature to that shown in Fig. 3.

Several L-amino acids are able to evoke marked membrane depolarisation of pancreatic acinar cells. This phenomenon is very different from the previously demonstrated electrophysiological effects of pancreatic secretagogues[13-16] in that the latency is much shorter and the null (reversal) potential much more positive (+40, rather than −15 mV). The clear resistance reduction (Fig. 2), together with the finding that the null potential has a value close to the calculated Na equilibrium potential, indicates that L-alanine, and several other amino acids, open up conductance pathways in the plasma membrane mainly permeable to Na.

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Induction of interferon in AKR mice by murine leukaemia viruses

Kenneth J. Blank

Department of Pathology, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19104

Donna Marie Murasko

Department of Microbiology, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129

A high percentage of AKR mice develop spontaneous leukaemia which has been shown to be associated with the early expression of ecotropic murine leukaemia virus (MuLV)[1] and the subsequent expression of xenotropic[2] as well as polytropic[3] MuLVs. Generally, mice infected with any one of several groups of viruses, including MuLVs, have been shown to produce interferon (IF)[4]. However, we report here that AKR mice produce no IF, despite that fact that infectious, endogenous MuLV is expressed in these mice from birth[5].

Three possible reasons for the lack of IF in AKR mice can be considered: (1) a genetic deficiency in their ability to produce IF in response to MuLV; (2) the particular MuLV produced by AKR mice is not an inducer of IF; (3) the chronic production of MuLV in AKR mice interferes in some way with the induction or production of IF. The last possibility is suggested by previous studies demonstrating that mice chronically infected with lymphocytic choriomeningitis virus produce substantially less IF in response to inoculation of Newcastle disease virus than do uninfected controls[6].

These three possibilities were examined in the experiments described in Table 1 legend. AKR, B10.K, BALB/c and NFS mice were infected with virus derived from preleukaemic AKR mice (AKR-P) or with other MuLVs. Sera were collected from these mice 6 and 24 h later and IF titres in the sera were measured by a modification[7] of the microplate method described by Havell and Vilček[8]. Mice from strains other than AKR were included in this study because, unlike AKR, they do not chronically produce endogenous, infectious ecotropic MuLV. In addition, NFS ($Fv\text{-}1^n/Fv\text{-}1^n$) mice were included to eliminate any possible effect of *Fv-1* (ref. 9), as N-tropic AKR-P virus does not replicate well in cells from B10.K or BALB/c mice because of their $Fv\text{-}1^b/Fv\text{-}1^b$ genotypes. The Gross virus (GV) and Friend virus (FV) used in these studies were N→NB tropic, and thus replicate equally well in mice from strains homozygous for $Fv\text{-}1^n$ or $Fv\text{-}1^b$.

The results shown in Table 1 indicate that there is no genetic deficiency in AKR mice to prevent production of IF and that chronic expression of AKR-P does not interfere with IF production in AKR mice, as these mice produce IF after inoculation with GV or FV. It seems, however, that AKR-P virus does

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Table 1 Induction of interferon in different mouse strains by various murine leukaemia viruses

Virus	Time (h)	Titre of interferon in: AKR	B10.K	BALB/c	NFS
—	0	<15	<15	<15	<15
GV	6	<15	<15	<15	<15
AKR-P	6	<15	<15	<15	<15
FV	6	20	30	20	<15
BALB/c ext.	6	<15	<15	<15	<15
GV	24	720	180	120	360
AKR-P	24	<15	<15	<15	<15
FV	24	420	600	240	260
BALB/c ext.	24	<15	20	<15	<15

AKR/J mice (Jackson Laboratory), aged 2–3 months, and C57BL/10-H-2^k (B10.K), BALB/c or NFS (bred in our colony) were inoculated intraperitoneally with 0.2 ml of various virus preparations. Virus preparations were 10% cell-free extracts of spleen from: leukaemic BALB/c mice infected with N → NB tropic Gross virus (GV) or N → NB tropic Friend virus (FV); preleukaemic AKR mice (AKR-P); or normal BALB/c. Virus titres (plaque-forming units per ml) determined in the XC assay[9] were: FV, 2.2×10^6; GV, 4×10^4; AKR-P, 6×10^4; BALB/c, 2×10^0. Mice were bled at 6 and 24 h after inoculation of virus. Sera of two or three mice per group were pooled and assayed for the presence of IF by the microplate method of Havell and Vilček[7], as modified by Armstrong[8]. Briefly, 50,000 L cells were added to wells containing serial twofold dilutions of sera. The cells were challenged 24 h later with encephalomyocarditis virus at a multiplicity of 0.2. Changes in cytopathology were recorded 24 h later. Titres were expressed as the highest dilution which protected half of the cells against virus-induced cytopathology. This was expressed as 1-ml volumes and corrected against mouse IF reference standard G002-904-511 distributed by the Reference Reagents Board of the National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. The values given are the average of results from two sets of assays. Neutralisation of IF activity of the samples was as described by Paucker *et al.*[13]. The IF induced by GV and FV in all strains of mice was totally neutralised by sheep anti-type 1 mouse fibroblast IF antiserum.

not induce IF, because none of the strains of mice studied produced detectable amounts of IF after inoculation with this virus. As the titre of AKR-P virus was similar to that of the GV preparation as measured in the XC assay[10] (see Table 1 legend), the lack of IF induction by AKR-P virus cannot have been caused by differences in the amount of virus inoculated.

One possibly relevant distinction between the non-IF-inducing AKR-P virus and the IF-inducing GV is that the latter was derived from leukaemic AKR mice. It was therefore necessary to determine whether a recent virus isolate from leukaemic AKR mice (AKR-L) was capable of inducing the production of IF. The results shown in Table 2 indicate that the AKR-L virus preparation, similar to the AKR-P preparation, does not induce IF production in AKR (*Fv-l*^{n}/*Fv-l*^{n}) or DBA/2 (*Fv-l*^{n}/*FV-l*^{n}) mice. The difference in abilities of GV and FV on the one hand, and of the AKR-P and AKR-L viruses on the other, to induce IF, may reflect a change in the GV and FV during laboratory passage. It is interesting that, although live AKR viruses do not induce IF, a previous study has demonstrated that exogenous IF is effective in inhibiting endogenous MuLV production in AKR mice[11].

Table 2 Induction of interferon by virus from leukaemic and pre-leukaemic AKR mice

Virus	Time (h)	Titre of interferon in AKR	DBA/2
—	0	<15	<15
AKR-P	24	<15	<15
AKR-L*	24	<15	<15
GV	24	80	135
FV	24	150	90

For techniques, see Table 1 legend. Titres are the average of the results from two sets of assays.

* 10% Cell-free extract of spleen from leukaemic AKR mouse (AKR-L). Virus titre as determined in the XC assay: 3×10^4 ml^{-1}.

It is thought that during gestation of AKR mice a small number of cells begin to express virus spontaneously[12]. Virus produced by these cells then infects other cells which in turn produce virus, and a cascade effect results. If AKR virus did induce IF production by infected cells, the progression of the infection might be aborted because of the protective effect of IF. Thus, it is interesting to speculate that in selecting for a strain of mice with a high incidence of spontaneous leukaemia, Furth may have selected for a strain (AK) which produces an MuLV that, although sensitive to IF, does not induce IF.

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Induction of secondary cytotoxic T lymphocytes by liposomes containing HLA-DR antigens

Steven J. Burakoff*, Victor H. Engelhard† James Kaufman† & Jack L. Strominger†

* Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115
† The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

It is evident that the gene products of the major histocompatibility complex (MHC) have a crucial role in the induction of cytotoxic T lymphocytes (CTLs) and as target antigens for CTLs[1–6]. Assessment of the precise molecular requirements for CTL induction and recognition, however, is difficult when intact cells are used as targets. Recently, in several model systems, it has been demonstrated that, after detergent solubilisation, cell-surface antigens can be isolated that retain the ability to stimulate a secondary CTL response. CTL stimulating activity has been isolated by detergent solubilisation from murine tumour cell plasma membranes[7,8] which co-purified with H-2 antigens through lentil lectin affinity chromatography and gel filtration[9,10]. Highly purified HLA-A and HLA-B antigens, from the human lymphoblastoid cell line JY, have been isolated and incorporated into well characterised liposomes[11]. Spleen cells from mice primed to JY cells developed cytolytic activity, specific for JY targets, after co-culture with these HLA-liposomes[12]. In these experiments it was observed that membranes which had been solubilised with deoxycholate and then dialysed to remove the detergent (reconstituted membranes) always stimulated greater cytolytic activity than did HLA-liposomes. At the time it was suggested that this disparity might reflect the loss of other antigens involved in CTL stimulation as a consequence of HLA-A and HLA-B purification. Data are presented here which demonstrate that other antigens are indeed able to induce secondary CTLs, independently of the HLA-A and HLA-B antigens. The results suggest that this activity is present on HLA-DR-associated molecules.

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L/6 mice were primed intraperitoneally with 2×10^7 Y cells 1–2 months and again 1 week before *in vitro* culture [12]. Spleen cells were then isolated and co-cultured for 5 days with various liposome preparations. Cytolytic activity was assessed by incubation of the effector cells with ^{51}Cr-labelled target cells. Reconstituted membranes were prepared by solubilisation of JY membranes in 0.5% deoxycholate, centrifugation at 100,000*g* for 1 h, and dialysis of the supernatant[11]. The 100,000*g* supernatant contained 80% of the membrane protein and 90% of the membrane phospholipid. Reconstituted membranes were selectively depleted of certain proteins by passage of the detergent-solubilised supernatant through anti-β_2-microglobulin–Sepharose or lentil lectin–Sepharose columns before dialysis. Material specifically eluted from the columns was reconstituted by dialysis after addition of JY lipid. Liposomes containing purified HLA-A and HLA-B antigens were prepared as previously described[11]. Briefly, the dried lipid was dispersed together with the purified protein in 0.5% deoxycholate, and the detergent was then removed by dialysis. Data indicate that the material eluted from the lentil lectin column also becomes associated with phospholipid vesicles (K. Parker, V.H.E. and J.K., unpublished). Equivalent amounts of dialysed material (based on A_{280} and phospholipid content) were centrifuged at 100,000*g* for 1 h and resuspended in a volume corrected for the amount of dilution which occurred on the columns. The pellets generally contained 80–90% of the detergent-solubilised phospholipid and protein[13].

The CTL-stimulating activity of these various preparations was assessed on JY targets at an effector: target ratio of 100:1 (Table 1). Reconstituted membranes stimulated far greater cytolytic activity than did liposomes containing only purified HLA-A and HLA-B antigens (compare samples 1 and 5). In addition, the flowthrough from the anti-β_2 column (sample 3) demonstrated CTL-stimulating activity. As assessed by inhibition of complement-dependent cytotoxicity, this preparation contained less than 0.005% of the original amount of the HLA-A and HLA-B antigens. However, passage of solubilised membranes over a lentil lectin column was sufficient to remove stimulating activity entirely.

Table 1 Only a portion of CTL-stimulating activity is removed by anti-β_2-microglobulin

Sample	HLA (μg ml^{-1})	% Lysis of JY
1. Reconstituted membranes	20	52
2. One pass over anti-β_2 column	0.5 (0.2%)	20
3. Two passes over anti-β_2 column	0.001 (0.005%)	16
4. One pass over lentil lectin column	0.9 (4.4%)	0
5. HLA-A and HLA-B liposomes	20	26

Fractions were prepared by solubilisation of 2.5 mg of JY membranes in 2 ml 10 mM Tris, 0.14 M NaCl and 0.5% deoxycholate, *p*H 8.0, followed by centrifugation at 100,000*g* for 1 h. The supernatant was either dialysed immediately against 10 mM Tris, 0.14 M NaCl and 5 mM $CaCl_2$, *p*H 7.8, or passed through anti-β_2-microglobulin–Sepharose or lentil lectin–Sepharose columns (0.5 ml each). The anti-β_2-microglobulin column had been equilibrated in solubilisation buffer, and the lentil lectin column was equilibrated in 10 mM Tris, 0.14 M NaCl, 0.1% deoxycholate, 75 μM $CaCl_2$ and 75 μM $MnCl_2$, *p*H 8.0. The flowthrough fractions were monitored by A_{280}, and fractions representing at least 85% of the recovered A_{280} were pooled. The extent of dilution of this material compared with the starting material was assessed by phospholipid analysis according to Ames[26]. The pooled material was then dialysed as described above. After dialysis all fractions were centrifuged at 100,000*g* for 1 h and resuspended in 1 ml 10 mM Tris, 0.14 M NaCl and 5 mM $CaCl_2$, *p*H 7.8, per ml of original detergent-solubilised supernatant. Aliquots of 50 μl was added to each 2-ml culture well. Lysis is expressed as per cent specific release of ^{51}Cr (ref. 2).

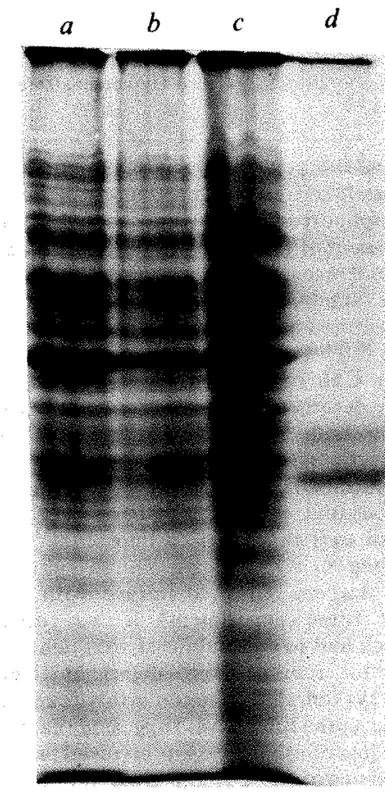

Fig. 1 12% SDS-polyacrylamide gel electrophoresis patterns of fractions from Table 2. Lane *a*, detergent-solubilised membrane supernatant; lane *b*, anti-β_2-microglobulin flowthrough; lane *c*, lentil lectin flowthrough; lane *d*, lentil lectin eluate.

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Table 2 presents evidence that solubilised membrane proteins which flow through the anti-β_2 column and specifically bind to lentil lectin can stimulate CTL activity when incorporated into liposomes. SDS-polyacrylamide gel electrophoresis of fractions from Table 2 shows that although the flowthrough material from the anti-β_2-microglobulin and lentil lectin columns contained most of the protein bands present in the reconstituted membranes, the lentil lectin eluate consisted primarily of two polypeptide chains of molecular weights 29,000 and 34,000, with no detectable HLA-A and HLA-B band (Fig. 1). These polypeptides have previously been shown by various immunological and protein chemical criteria to correspond to the HLA-DR alloantigens, which are the human analogues of the murine Ia antigens[14,15]. Although other minor protein bands are present in the lentil lectin eluate, the presence of HLA-DR antigens as the major component of this material, coupled with previous reports of Ia- or HLA-DR-specific allogeneic CTL, suggest that these antigens represent the non-HLA-A and -B CTL-stimulating activity seen here.

We are currently investigating the specificity of the CTLs induced by these HLA-DR-containing liposomes. It is possible that CTLs are induced with specificity to HLA-A and HLA-B antigens or that these liposomes induce CTLs with specificity for HLA-DR antigens. These possibilities were tested as follows:

Table 2 The CTL-stimulating material which flows through the anti-β_2-microglobulin column can be specifically bound to and eluted from lentil lectin

Sample	% Lysis of JY
1. Reconstituted membranes	54
2. Two passes over anti-β_2 column	38
3. Above passed through lentil lectin column	4
4. 2% α-methyl mannoside eluate of lectin column	31
5. HLA-A and HLA-B liposomes	24

The procedure was the same as that described in Table 1 legend, except that the detergent-solubilised material was passed through the lentil lectin column only after passage through the anti-β_2-microglobulin column. In addition, the lentil lectin eluate was reconstituted after addition of 1 mg ml^{-1} JY lipid.

CTLs were induced with liposomes containing either HLA-A and HLA-B or HLA-DR. These CTLs were assayed on Daudi target cells, which are HLA-A and HLA-B negative but HLA-DR positive[16]. Only CTLs induced with HLA-DR liposomes lysed Daudi, suggesting that these CTLs have specificity for HLA-DR antigens (Table 3). We confirmed that the cytolytic activity observed on Daudi targets resided in the T-cell population by two criteria: cytolytic activity was eliminated by treatment of cells with anti-Thy 1.2 serum plus complement, and the effluent cells from nylon wool column passage (which removed ≥95% Ig$^+$ cells) retained cytolytic activity (data not shown). Preliminary experiments indicate that an antiserum raised against the highly purified HLA-DR complex (anti-p 29, 34)[17] and which recognises only these two chains by immunoprecipitation[14] blocks target cell lysis by CTLs stimulated with HLA-DR-containing liposomes.

Table 3 CTL induced with HLA-DR containing liposomes lyse Daudi target cells

Stimulator	Target JY	Target Daudi
HLA-A and HLA-B liposomes	66	5
HLA-DR liposomes	78	55

Spleen cells from C57BL/6 mice primed with JY were stimulated with either HLA-A and HLA-B or HLA-DR liposomes. These CTL were then assayed after 5 days on either JY or Daudi targets.

There have been several reports that CTLs can be generated to mouse allogenic Ia antigens[18-20]. It has also been observed that these Ia antigens are recognised by CTLs independently of H-2K and H-2D antigens[21,22]. Recently, several studies have shown that CTLs were generated in human mixed lymphocyte cultures between donors differing at the HLA-D/DR but not HLA-A and HLA-B loci[23-25]. These CTLs only lysed targets bearing HLA-DR region antigens (monocytes and Epstein-Barr virus-transformed peripheral blood lymphocytes).

In conclusion, non-HLA-A and -B cell surface antigens have been isolated from JY cells that are able to stimulate primed xenogeneic mouse spleen cells to exhibit cytolytic activity. These antigens seem to be HLA-DR antigens, the human equivalent of the mouse Ia antigens, and preliminary data suggest that the CTLs induced are specific for HLA-DR antigens. Further studies are in progress to define the specificity of the CTLs induced and to determine whether other biological functions are associated with these isolated HLA-DR antigens.

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The joining of V and J gene segments creates antibody diversity

Martin Weigert, Robert Perry & Dawn Kelley

The Institute for Cancer Research, Fox Chase Cancer Center,
Philadelphia, Pennsylvania 19111

Tim Hunkapiller, James Schilling & Leroy Hood

California Institute of Technology, Division of Biology, Pasadena,
California 91125

The variable regions of mouse kappa (κ) chains are coded for by multiple variable (V) gene segments and multiple joining (J) gene segments. The V_κ gene segments code for residues 1 to 95; the J_κ gene segments code for residues 96 to 108 (refs 1–3). This gene organisation is similar to that encoding the V_λ regions[4]. Diversity in V_κ regions arises from several sources: (1) there are multiple germ-line V_κ gene segments and J_κ gene segments; (2) combinatorial joining of V_κ gene segments with different germ-line J_κ gene segments; and possibly, (3) somatic point mutation, as postulated for V_λ gene segments[5]. Also, from a comparison of the number of germ-line J_κ gene segments and amino acid sequences, it has been suggested that J_κ region sequences may be determined by the way V_κ and J_κ gene segments are joined[2,3]. This report supports this model by directly associating various J_κ sequences with given J_κ gene segments.

The number of germ-line J_κ gene segments has been estimated in two ways. A region of DNA containing J_κ gene segments has been characterised by DNA sequence analysis and was found to include five J_κ (or J_κ-like) gene segments, only four of which have been found to be expressed as J segments[2,3]. Additional J_κ gene segments have not been found, although their presence cannot be excluded rigorously. Alternatively, the number of J_κ gene segments may be determined by counting the number of distinctive RNA precursor patterns associated with different κ chain mRNAs[6]. Since the κ-mRNA precursors contain the intervening sequence between the J_κ and C_κ segments, they have a characteristic size depending on which J segment is used, the largest precursor corresponding to the J_κ segment most distal to the C_κ gene and the smallest to the most proximal J_κ segment. On this basis the RNA precursor patterns for 20 different myeloma tumours were classified into four different categories, corresponding to the four different J_κ gene segments that these chains use. The distances between the C_κ gene and the various J_κ gene segments deduced from the analysis of precursor patterns in NZB plasmacytomas agree with the distances determined by restriction mapping and DNA sequence analysis of BALB/c germ-line DNA. A previously suspected discrepancy[6] has been resolved by a more accurate calibration of the mobility data.

We have analysed κ chains of the V_κ 21 group from the NZB mouse strain. To date ten different J_κ amino acid sequences have been observed in twenty V_κ 21 examples (Fig. 1). Thus the

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Fig. 1 J segments associated with V_κ21 chains from NZB myeloma antibodies. J segments are numbered according to their processing category[6]. This is based on the relative sizes of the κ-mRNA precursors, as determined by gel-electrophoretic analysis of poly(A)$^+$ nuclear RNA. These precursors are approximately 5.3, 5.0, 4.4 and 4.1 kilobases for categories I, II, III and IV, respectively. The amino-terminal sequences (40 residues) of PC8701, PC8982 and PC10916 were determined on a modified Beckman sequenator as described previously[11] and were found to be representatives of the V_κ21 subgroups, D, C and C, respectively. The J segment sequence of these κ chains were determined as follows: each κ chain was subjected to mild acid cleavage[12] of the aspartylproline between residues 94 and 95. The peptide mixture was sequenced without separation after the amino-terminal peptide was blocked by addition of fluorescamine[13]. The rest of the sequences are taken from ref. 1. The germ-line J segments are defined as described in the text. J_1, J_2, J_3 and J_4 are coded for by the BALB/c embryo J gene segments termed J_1, J_2, J_4 and J_5 in ref. 2 or those termed J_5, J_4, J_2 and J_1 in ref. 3. The PC2413 J segment may be coded for by a germ-line J gene (or variant of a J gene) unique to the NZB mouse.

	Sequences of J segments associated with V_κ21 segments	Frequency	Processing category
Germline	96 107		
J_1	W T F G G G T K L E I K R	5/20	I
J_2	Y————————————	3/20	II
J_3	F————S————————	2/20	III
J_4	L————A————————L——	4/20	IV
J?	W——————————D————E——	1/20	I
Variant			
J_1^* PC6684	R————————————	1/20	I
J_1^* PC7940	P————————————	1/20	I
J_2^* PC7132	Ⓟ Y————————————	1/20	II
J_3^* PC8701	[ ]————S————————	1/20	III
J_4^* PC8982	L————A————E————L——	1/20	IV

number of J_κ sequences appears to be greater than the number of germ-line J_κ gene segments. There are three types of J_κ sequence. First, four distinct types (J_1, J_2, J_3 and J_4) correspond to those coded for by four of the germ-line J_κ gene segments in BALB/c DNA. Each of these J_κ sequences has been observed two or more times in the NZB V_κ21 regions (Fig. 1). These J_κ sequences also have been observed in BALB/c κ chains[7] indicating that NZB and BALB/c germ-line J_κ gene segments code for the same J_κ regions. A second type of J_κ sequence differs by a single residue from those of the germ-line J_κ gene segments; these are termed variant J_κ sequences. There are five J_κ sequences of this type and each has been observed only once.

There are two possible explanations for the variant J_κ sequences: (1) they arise from somatic diversification which occurs at the junctional region during the joining of V_κ and J_κ gene segments or (2) they are encoded by additional germ-line J_κ gene segments that are absent from or have not yet been detected in the BALB/c genome. The former model predicts that the precursors of the mRNAs coding for these variant J_κ segments would belong to a category typical of one of the four germ-line examples; the latter model predicts a unique mRNA precursor for each variant. A third type of J sequence which occurs in the exceptional PC2413, differs from the other germ-line J_κ gene segments by at least two nucleotide substitutions.

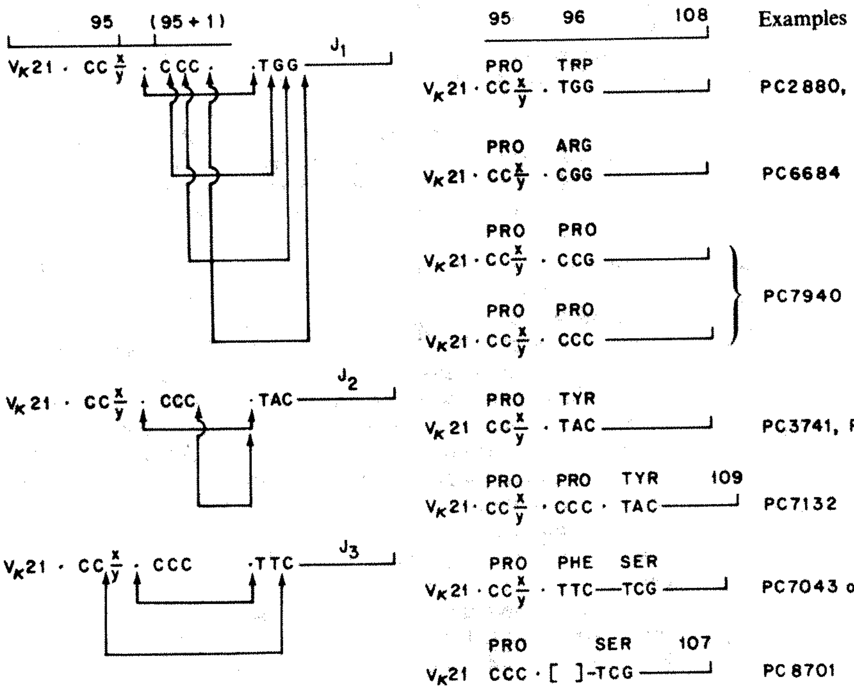

Fig. 2 Fusion of V_κ21 genes and J gene segments. The fusion can occur to join V and J gene segments exactly to form a contiguous V–J gene. Such genes will have germ-line J region sequences as in the case of PC2880, PC3741 or PC7043 and other examples[1]. Fusion to J genes might include nucleotides in the intervening sequence (95+1). If this codon is CCC for all V_κ21, then the variant sequences, PC6684, PC7940 and PC7132 can be explained by fusion to a J gene (PC7132), within a J gene (PC6684 and PC7940) or within a V gene (PC8701).

Differences of this extent raise the issue of whether the PC2413 J_κ sequence might be encoded by a fifth germ-line J_κ gene segment in the NZB mouse.

An analysis of the RNA processing patterns for the tumours producing κ chains with variant J_κ sequences indicates that each pattern belongs to one of the four categories characteristic of germ-line J_κ gene segments (Fig. 1). Thus it appears that these variant J sequences arise during the process of V_κ–J_κ joining.

Knowing the size of the mRNA precursor for a J_κ variant allows us to infer the kind of somatic mutation that could have produced the variant sequence. For example, both PC6684 and PC7940 mRNAs exhibit the J_1 type prescursor. Their J segments differ from the germ-line J_1 segment at position 96, presumably due to modifications of the tryptophan codon 96. PC7132 arose from the insertion of a proline codon before the J_2 gene segment. PC8701 resulted from a deletion of the phenylalanine codon at the beginning of J_3 gene segment. In addition to this junctional diversity, random point mutations either in the germ line or during somatic differentiation may occasionally occur (for example, PC8982).

The κ-mRNA precursor in PC2413 appears to belong to the category characteristic of the germ-line J_1 segment. Thus, if the J sequence of this tumour is actually encoded by a unique NZB germ-line J_κ segment, thus segment would have to be located very close to the J_1 segment, or alternatively, be associated with a second, as yet unidentified, C_κ gene.

The nature of most J_κ variants suggests that they may be due to variations arising from the V–J joining process because the substitutions, mutations, insertions or deletions generally occur at the beginning of J_κ segments. These variations may be explained by two base substitutions (for example, tryptophan to proline) as well as the insertion or deletion of codons. Thus this variation does not appear to arise merely by single-base somatic mutation. Leder and Tonegawa and their coworkers have recently observed identical inverted repeat sequences at the 3′ end of the V gene segment and at the 5′ end of the J gene segment. They have hypothesised that these inverted repeats may form paired stem structures that juxtapose the V and J gene segments and that recombination may occur across the base of this stem at slightly different points to generate V–J junctional diversity (refs 2 and 3 and Fig. 2). This site-specific recombinational model appears to explain the V–J junctional diversity that we have noted in the $V_\kappa 21$ chains.

By whatever mechanism V gene segments are translocated to J gene segments, a continuous DNA sequence coding for the entire V region must be generated[4]. Since all mouse κ chains studied to date have proline at residue 95 (CC_y^x), a V_κ gene segment translocation to the J_1 gene segment (tryptophan (TGG) at residue 96) will result in the contiguous DNA sequence, CC_y^x TGG at the V–J joining site (Fig. 2). Such a translocation will create a V_κ gene segment that is associated with the J_1 germ-line gene segment. This type of translocation explains five out of the twenty $V_\kappa 21$ regions observed (Fig. 1). Likewise, similar translocations between $V_\kappa 21$ gene segments and the J_2, J_3 and J_4 gene segments can explain the nine additional examples of germ-line J segments (Fig. 1).

The variant J segments may arise by recombination within rather than between the V gene segment codons for positions 95 or 95 + 1 and their counterparts in the J gene segments (Fig. 2). Codon 95 + 1 is CCC in several V_κ gene segments[2,8]. If the (95 + 1) codon is CCC for all $V_\kappa 21$ gene segments, the type of variation that may occur at the V–J joining site is limited. Amino acid substitutions at residue 96 for a $V_\kappa 21$–J_1 translocation can only be arginine (CGG) or proline (CCG) (Fig. 2). An amino acid insertion would result in an extra proline codon before the J gene segment. In addition, deletions of residue 96 could occur. As shown in Fig. 2, the variants observed so far fit the predictions of this model. If there is variation at the (95 + 1) codon, a greater variety of substitutions could occur at residue 96[3]. Obviously similar variation could occur in the (96 − 1) codon of the J gene segment. Additional analyses of $V_\kappa 21$ chains are under way to define more completely the amino acid sequence variability at the V–J junctional site.

In our survey of $V_\kappa 21$ chains, the frequency of κ chains expressing variant J segments relative to those expressing germ-line J segments is high (5 out of 20). This frequency could be high because the N-terminus of the J_κ segment is important for combining-site size and shape[9] and antigen selection of specific antibodies amplifies mutations that occur at a low frequency. (The uniformity of J_λ segments[10] may mean that variation in this region does not affect the complementarity of antibodies with λ chains.) Alternatively, this frequency may be a true reflection of the rate of variation by this mechanism.

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Translation of globin messenger RNA by the mouse ovum

R. L. Brinster, H. Y. Chen, M. E. Trumbauer & M. R. Avarbock

Laboratory of Reproductive Physiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

It has been demonstrated that the *Xenopus* oocyte can translate rabbit haemoglobin messenger RNA (mRNA) following microinjection of the message into the cell[1]. The *Xenopus* oocyte has since been shown to be capable of translating a variety of messenger RNAs from different species[2–4]. This system has proved useful in understanding the mechanism of message translation and has also provided information about the translation capability of the *Xenopus* oocyte[5,6]. Several other cell types, including HeLa cells and fibroblasts, can also translate exogenous message injected into the cell[7,8]. However, there have been no reports of injection of mRNA into oocytes or fertilised one-cell ova of mammalian species. Nevertheless, the latter system could be of considerable use in studying the processing of exogenous messages in a mammalian system undergoing development, as well as providing insight into the way the early embryo processes injected messages and the protein products of such messages. We report here the results of injecting message into the fertilised one-cell mouse ovum and show that both mouse and rabbit globin mRNA are translated in this system.

Fertilised one-cell mouse ova were collected following superovulation from hybrid C57 × SJL females on the day of the vaginal plug by techniques described previously[9]. The ova were collected in Brinster's medium for ovum culture (BMOC-2, see ref. 9) and the cumulus cells removed with hyaluronidase (300

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$^{-1}$) in phosphate-buffered saline[9]. After washing to remove cumulus cell debris, the ova were maintained in BMOC-2. Purified mRNA for these experiments was prepared by the method of Gorski *et al.*[10] except that polysomes were dissociated with SDS and applied directly to oligo(dT)-cellulose as described by Krystosek *et al.*[11]. For injection, the ova were placed on a depression slide and held with a blunt pipette as shown in Fig. 1. The tip of the injector pipette was filled with a solution of mRNA in water (1 mg ml^{-1}) and a small amount of solution was injected into each ovum. The average volume injected was determined by injecting ^{3}H-inulin into several groups of ova and measuring the radioactivity found in each ovum. On the basis of these measurements, the average volume injected into each ovum was estimated to be 13 pl. Some of the ova underwent lysis during the 15–30 min after removal of the injector pipette and release of the ovum from the holder. The survival rate was 30–40%, and it was found that injection of the ova in medium containing cytochalasin B (5–10 μg ml^{-1}) approximately doubled the chance of survival. Each group of 20–40 injected ova was placed in BMOC-2 containing a mixture of five ^{3}H-labelled amino acids (leucine, lysine, phenylalanine, proline, tyrosine; specific activities 75–120 Ci mmol^{-1}; Amersham Searle) for 15–17 h, during which time approximately one third of the one-cell ova divided into two cells. Generally, about 80% of one-cell ova will cleave during this period, but the high level of radioactivity necessary for labelling decreased the number of ova undergoing cleavage. After labelling, the ova were washed three times in media containing non-radioactive amino acids, placed in a 6 × 60 mm tube and stored at −70 °C. To determine whether globin had been synthesised from the injected message, non-radioactive globin marker was added to the tube, and the proteins were separated by two-dimensional high-resolution electrophoresis[12].

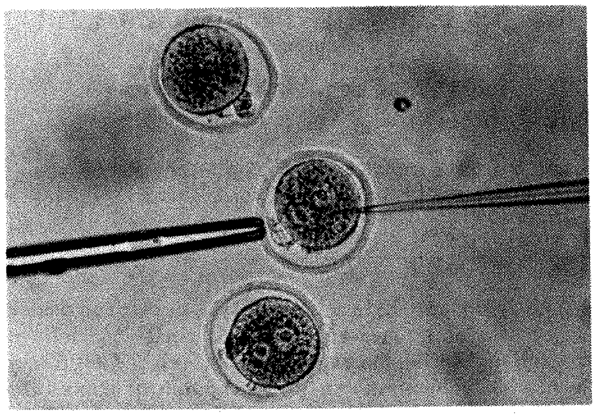

Fig. 1 Injection of one-cell mouse ovum with mRNA. The ovum was held with a blunt suction pipette (5 μm i.d., 20 μm o.d.). The o.d. of the injector pipette was 2–3 μm, and the tip was sharpened either by chipping or grinding. The ova were maintained on a depression slide in culture medium under silicone oil. The injection solution drop, from which the injector pipette was filled, was adjacent to the medium containing the ova. The technique was similar to that used for injecting cells into blastocysts[15].

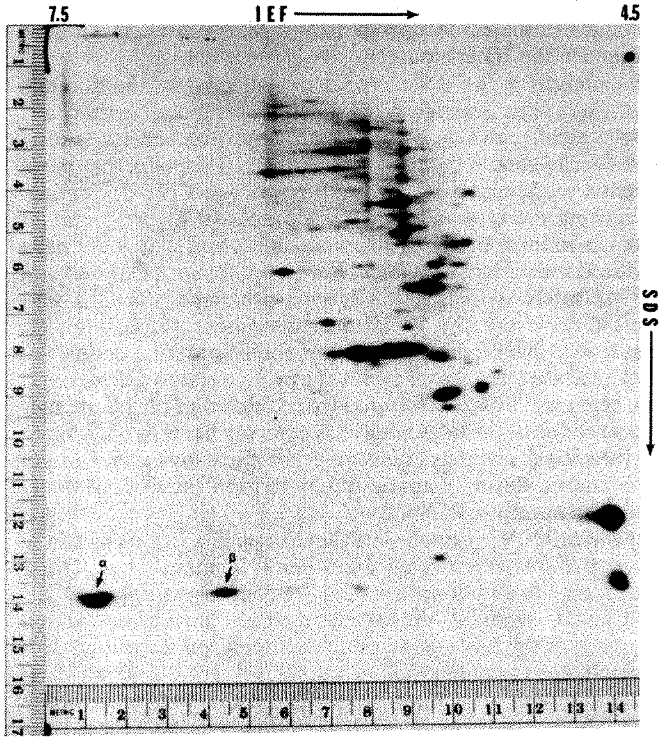

Fig. 2 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with mouse globin mRNA and incubated in ^{3}H-labelled amino acids. Isoelectric focusing (IEF) was from left (*p*H 7.5) to right (*p*H 4.5) and SDS-polyacrylamide electrophoresis was from top to bottom. The technique used was basically that of O'Farrell, as previously described[12,16]. The IEF was carried out with *p*H 3–10 ampholines. Marker proteins on the right margin are lactoglobulin (top, MW = 18,400) and methaemoglobin (bottom, MW = 16,000). The two radioactive spots indicated by the arrows coincide with the position of the non-radioactive globin marker protein prepared from mouse blood. α and β indicate the positions of α- and β-chains of globin.

Figure 2 shows a typical fluorograph of the electrophoretic separation of synthesised proteins following injection of ova with mouse globin mRNA. Two major radioactive spots, indicated by arrows, co-migrated with non-radioactive globin marker prepared from mouse blood. Injection of message into mouse oocytes yields a similar pattern (unpublished). The other spots on the fluorograph are endogenous proteins. On control gels from ova not injected with mRNA there are no radioactive spots in the area of globin migration, indicating the absence of endogenous labelled protein in this area that might confuse the interpretation of the fluorographs (Fig. 3). When the non-radioactive markers are separated on isoelectric focusing (IEF) gels and stained for protein, each of the major spots is composed of two closely migrating but clearly distinct bands. This separation, although less apparent in the second dimension, can sometimes be seen in the radioactive spots such as the most basic of those in Fig. 2. No globin marker bands are present in the IEF gel at a more basic position than those shown in Fig. 2. The absence of such bands was confirmed by non-equilibrium *p*H gradient electrophoresis (unpublished observations); in this procedure the migration is from the acidic end and can be stopped at various positions before reaching the basic end[13]. Thus, the technique used for the egg proteins is capable of identifying the location of all the globin chains synthesised from the injected message.

When rabbit mRNA is injected into the ova, the pattern is slightly different. As shown in Fig. 4, only one major globin spot is present. This major spot seems to be composed of two sub-bands, similar to the situation for the mouse globin. However, because of the high level of radioactivity, it generally appears as one spot in the photographs of the fluorograph. By reference to purified globin markers for mouse α- and β-chain and for rabbit α- and β-chain, the large basic spot in Figs 2 and 4 was identified as α-globin for both the mouse and rabbit mRNA. The more acidic spot from the mouse globin mRNA is β-globin. No β-globin spot, which would be located in approximately the same position as the mouse β-globin, can be detected in Fig. 4.

However, other studies suggest that a low level of rabbit β-globin synthesis does occur (unpublished observations).

When ova were injected with either mouse or rabbit message at the one-cell stage, cultivated *in vitro* to the blastocyst stage (5 days) and then labelled with ^{3}H-amino acids, fluorographs showed no indication of globin synthesis. In fact, preliminary studies suggest that globin synthesis is not detectable 48 h after message injection. However, some globin is synthesised when labelling begins 24 h after message injection. A precise half life of the injected message can only be determined following more detailed investigation to identify the saturating level of message and the lag time before the message is active. However, it is clear that the mRNA half life in mouse ova is considerably shorter than that in *Xenopus* oocytes, where globin message has been shown to retain its activity for 2 weeks or longer[14]. Clearly, the cell type containing the message profoundly influences its stability. These studies do not indicate whether the message is degraded or is converted to a non-translatable form. Although the former possibility seems the more likely, the presence of masked message in the embryo makes the latter an intriguing possibility.

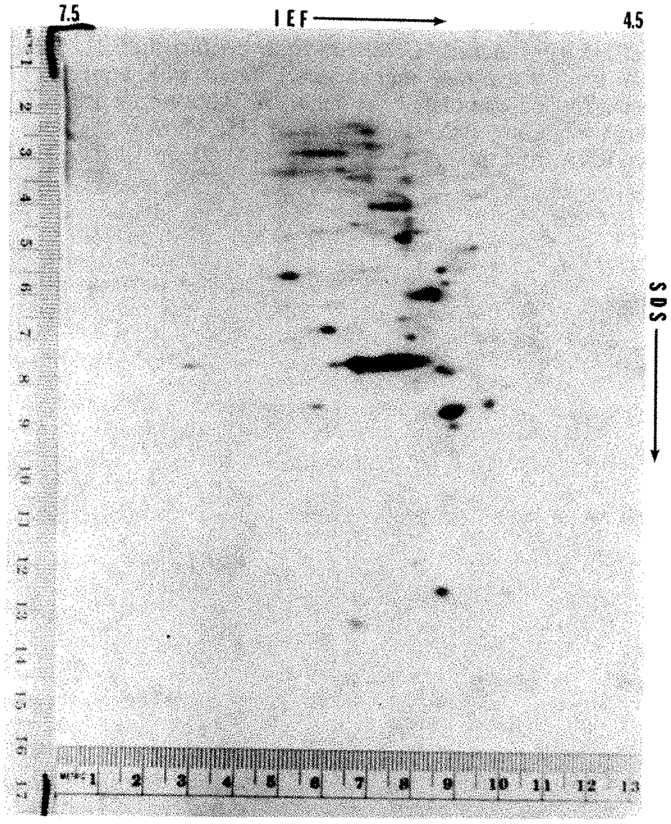

Fig. 3 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with water. Techniques used were similar to those for Fig. 2. No radioactivity appears in the area of globin migration.

These observations on the translation of globin message by the mouse ovum identify an important new system in which to study the activity of mRNA and the synthetic products of the message. Although having some similarities to the *Xenopus* oocyte, the system shows several differences. For instance, the mouse cells are mammalian rather than amphibian and are actively dividing rather than dormant or static as is the *Xenopus* oocyte. The unique characteristics of each system may provide different answers to the same question. The large difference found between the two cell types in the stability of injected globin message is one example. Another is the preponderance of synthesis of α- relative to β-chains in the mouse ovum; the relative synthesis is just the opposite in the *Xenopus* oocyte. The injection of exogenous messages into the mouse ovum should help in understanding message stability and activity in the developing mammalian embryo, as well as providing a means of studying translational activity and specific protein stability in the embryo.

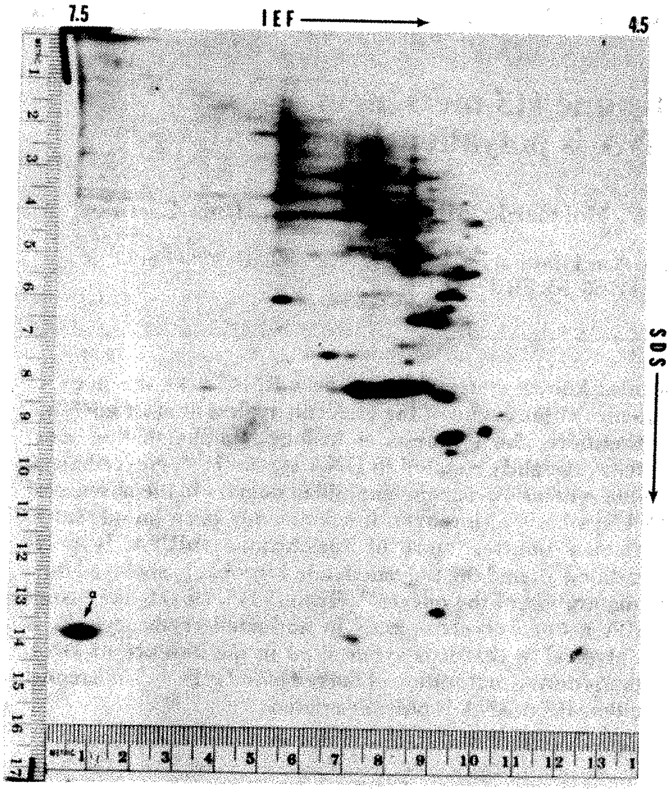

Fig. 4 Fluorograph of two-dimensional electrophoretic separation of proteins from 20 one-cell fertilised mouse ova injected with rabbit globin mRNA. Techniques used were similar to those for Fig. 2. The single radioactive spot indicated by the arrow coincides with the position of the non-radioactive globin marker protein prepared from rabbit blood. α Indicates the position of the α-chain of globin. No β-chain was detected on the fluorograph.

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Histone H5 messenger RNA is polyadenylated

H. V. Molgaard*, M. Perucho† & A. Ruiz-Carrillo‡

Max-Planck-Institut für Molekulare Genetik, D-1000 Berlin 33,
Ihnestr. 63–73, FRG

In most known systems[1,2], histone mRNA lacks the poly(A) sequence at the 3′ end of the molecule typical of most mRNAs. Furthermore, the synthesis of histones, unlike that of most proteins, is tightly coupled to DNA synthesis[3–5]. Nevertheless, histone synthesis occurs in amphibian oocytes in the absence of DNA synthesis[6]. Moreover, it has recently been found that in amphibian oocytes most of the histone mRNA is polyadenylated[7–9], and the polyadenylate is probably removed during maturation of the oocyte[10]. Histone H5, an H1-like tissue-specific histone occurring only in nucleated erythrocytes[11], is also atypical in that it is synthesised in the absence of DNA synthesis during maturation of the red blood cells[12,13]. We report here that H5 mRNA is polyadenylated.

RNA from anaemic chicken blood was enriched in H5 mRNA by immunoprecipitation of isolated polysomes[14], essentially as described by Groner *et al.*[15]. In one experiment polysomes were isolated and immunoprecipitated as described by Scott & Wells[16]. The result of passing total polysomal RNA, enriched for H5 mRNA, over poly(U)-Sepharose is shown in Fig. 1*a*. In this sample there is little evidence of translatable H5 mRNA in the unbound material (lane 4) and only a trace in the 5% formamide eluate (lane 5). The bulk of the translation product, which migrates at the same mobility as *in vitro*-labelled H5, occurs in the translation product of the fraction eluted by 90% formamide (lane 6). In Fig 1*b* a similar result is shown with a different sample of RNA which had been further purified by size fractionation on a sucrose gradient, and subjected to oligo(dT) cellulose chromatography. Although a small fraction of the H5 mRNA activity does not bind to oligo(dT) cellulose (lane 3) most of the translatable activity appears in the bound fraction eluted with distilled water (lane 5). To ensure that the binding of H5 mRNA was not due to an interaction with the cellulose support we passed the RNA over oligo(dA) cellulose (Fig. 1*b* lanes 6–8). The H5 mRNA showed no affinity for this support (compare lanes 6 and 8).

In all these experiments the H5 mRNA behaved in much the same way as the contaminating poly(A)-containing globin mRNA. That the translation products migrating with the mobility of H5 were indeed H5 follows from the fact that: (1) only RNA from polysomes immunoprecipitated with antibodies specific for H5 stimulated the rabbit reticulocyte to produce protein migrating with the mobility of H5, which was absent when RNA from total polysomes was used; (2) the cell-free H5

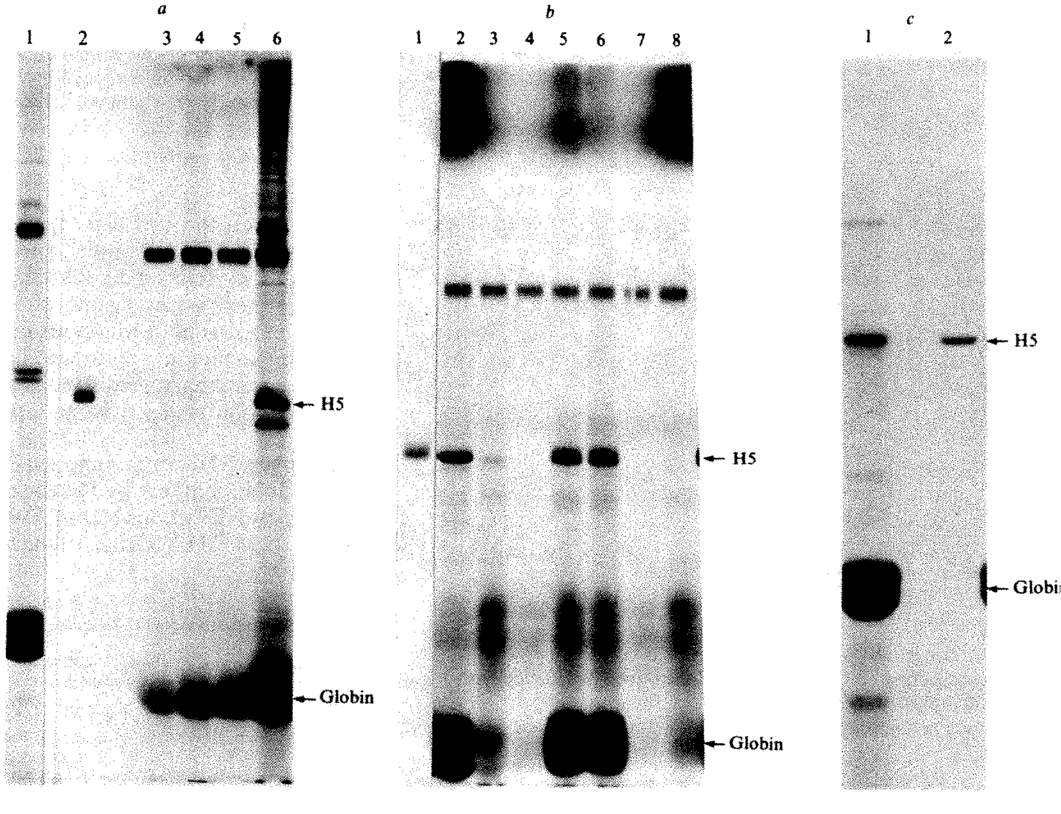

Fig. 1 H5 mRNA binding to poly(U)-Sepharose and oligo(dT)-cellulose. *a*, 7.5 μg of immature chicken erythrocyte polysomal RNA, phenol extracted[21] from polysomes enriched for H5 message by immunoprecipitation[16] was fractionated by poly(U)-Sepharose chromatography[22]. The unbound material, the 5% and 90% formamide eluates were, after ethanol precipitation, dissolved in 10 μl water. Aliquots (2 μl) of each of these fractions were analysed by translation in a rabbit reticulocyte cell-free translation system (NEN) using ^{35}S-L-methionine as label. Aliquots of the lysate were analysed on 15%–19% SDS polyacrylamide gels[23] and exposed by fluorography[24] on presensitised film[25]. *Lane 1*, total enriched polysomal RNA translated in the cell free system; *lane 2*, H5, labelled *in vitro*, by reductive methylation[26]; *lane 3*, no RNA added to the lysate; *lane 4*, polysomal RNA not bound in 0.2 M NaCl in ETS buffer (0.01 M Tris–HCl *p*H 7.5, 0.01 M EDTA, 0.2% SDS); *lane 5*, polysomal RNA eluted by 5% formamide in ETS; *lane 6*, RNA eluted by 90% formamide in ETS. *b*, immature chicken erythrocyte polysomal RNA was phenol extracted as above from polysomes[14] and immunoprecipitated as described by Groner *et al.*[15]. The RNA was purified by size fractionation on a sucrose gradient before being chromatographed on oligo(dT)-cellulose (T 3 Collaborative Research) and oligo(dA)-cellulose (T 6 Collaborative Research). The RNA was analysed by translation as above. The 0.4 M NaCl and 0.1 M NaCl solutions referred to below included 0.01 M Tris–HCl *p*H 7.4, 0.001 M EDTA, 0.5%, SDS. *Lane 1*, *in vitro* labelled H5; *lane 2*, RNA before oligo(dT)-cellulose chromatography; *lane 3*, RNA unbound in 0.4 M NaCl; *lanes 4 and 7*, RNA eluted with 0.1 M NaCl; *lanes 5 and 8*, RNA eluted with sterile water; *lane 6*, RNA not bound to oligo(dA)-cellulose in 0.4 M NaCl. *c*, Part of the RNA used in *b* was translated as described and the PCA-soluble translation products were precipitated by rabbit antibodies directed against H5 (manuscript in preparation). The labelled proteins were analysed as in *a*. *Lane 1*, an aliquot of the total translation assay; *lane 2*, the translation products precipitated by anti-H5.

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* Present address: King's College, Department of Biophysics, University of London, WC2, UK; † Present address: Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724; ‡ To whom correspondence should be addressed.

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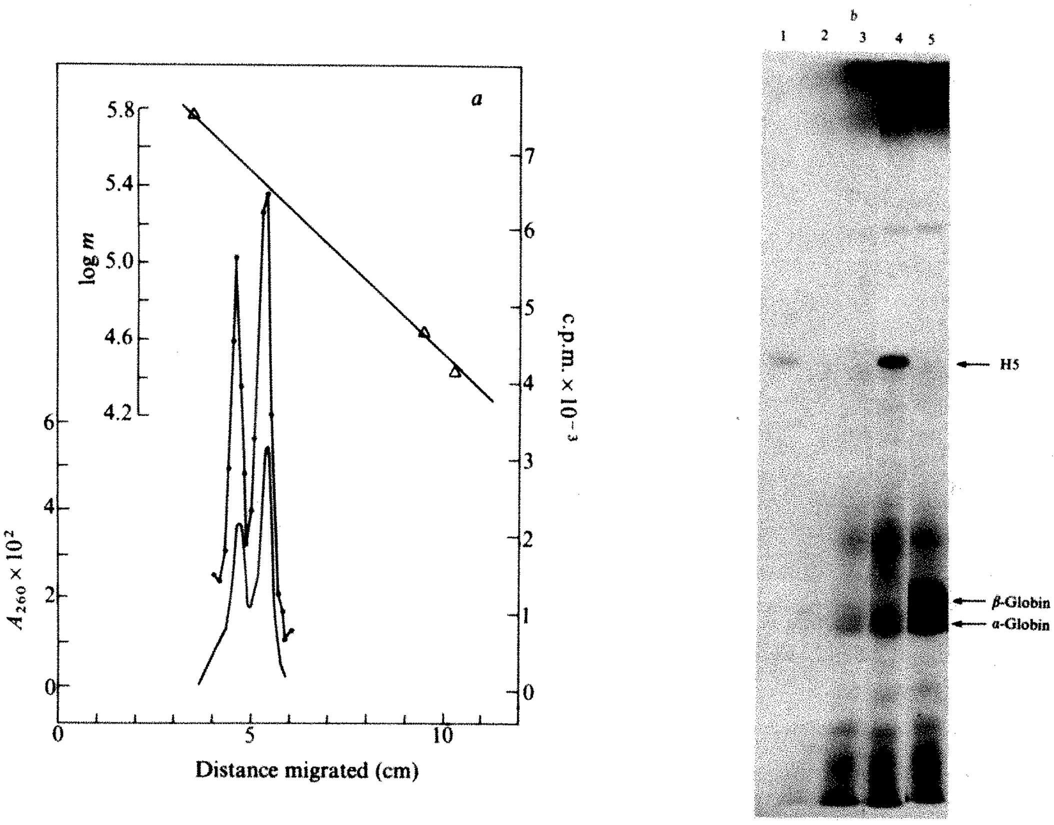

Fig. 2 Preparative electrophoresis of poly(A)$^+$RNA. RNA prepared as in Fig. 1*b* and size-fractionated by two cycles of sucrose gradient centrifugation was separated into poly(A)$^+$ and poly(A)$^-$ RNA by poly(U)-Sepharose chromatography. Both fractions were further analysed on 3.5% polyacrylamide, 99% formamide gels[27]. Labelled 16S and 5S *E. coli* rRNA (extracted from *E. coli* subunits provided by Dr R. Brimacombe) were included as size markers, and calf liver tRNA (Boehringer) as carrier and marker. After electrophoresis, the gels were washed for about 3 h in sterile water and scanned at 260 nm in a Gilford spectrophotometer. The gel was then frozen and sliced into 0.5-mm slices in a Mickle gel slicer. The slices were eluted in pairs in 0.5 ml of 0.6 M lithium acetate, 0.2% SDS[28], 5 μg ml^{-1} calf liver tRNA for about 18 h. After phenol extraction and ethanol precipitation, the eluates were dissolved in 10 μl sterile water. Aliquots (1 μl) were used for translation assays as described under Fig. 1*a* and for poly(U) hybridisation[29] with tritiated poly(U) (NEN). *a*, Absorbance at 260 nm (——); poly(U) hybridisation (—·—); 16S, 5S and 4S RNA markers (△). *b*, RNA samples separated by electrophoresis as in *a* were analysed by cell-free translation as in Fig. 1*a*. *Lane 1*, *in vitro* labelled H5 (ref. 26); *lane 2*, lysate without RNA added; *lanes 3–5*, translation products from RNA samples that migrated 4.1, 4.5 and 5.4 cm along the gel shown in *a*. The arrows mark the distances migrated by the respective marker proteins.

product was specifically solubilised by 5% perchloric acid which is known to selectively extract very lysine rich-histones[17]; and (3) all the protein-bound ^{35}S-methionine radioactivity extracted with 5% perchloric acid from cell-free assays was immunoprecipitated by purified rabbit anti-H5 antibodies, and comigrated with H5 on SDS electrophoresis of the immunoprecipitate (Fig. 1*c*, and manuscript in preparation).

To characterise further the H5 mRNA, total RNA from immunoprecipitated polysomes was fractionated by size by two sucrose gradient cycles and the poly(A)$^+$ RNA was separated by poly(U)-Sepharose chromatography and analysed by electrophoresis in polyacrylamide gels containing 99% formamide. Only two significant peaks were observed when the RNA gel was scanned at 260 nm (Fig. 2*a*). Translation of fractions eluted from the gel showed a peak of H5 mRNA activity in the more slowly migrating zone (Fig. 2*b*, lane 4) and mainly β-globin in the faster zone (Fig. 2*b*, lane 5). No other prominent translation products were observed.

For additional confirmation of polyadenylation of the H5 mRNA, peak fractions of the eluted RNA were hybridised with tritiated poly(U) (Fig. 2*a*). The more slowly migrating zone showed a degree of hybridisation similar to or greater than that of the globin component. The optical density, the poly(U) hybridisation and the translational activity coincided closely in both peaks. This result (Fig. 2*a*) leads to an estimated molecular weight of 3.38 (±0.15) × 10^5 for the polyadenylated H5 mRNA, that is, a length of about 1,000 nucleotides. A parallel electrophoresis of chicken globin mRNA gave two partly resolved peaks of molecular weights 2.34 × 10^5 and 2.14 × 10^5 for the β- and α-chains respectively, in good agreement with the data of Crawford *et al.*[18], but significantly higher than the molecular weights found by Longacre & Rutter[19].

If the H5 mRNA is polyadenylated then it should be possible to isolate and measure the length of the poly(A) since this sequence is relatively insensitive to RNase A and RNase T1.

Aliquots of fractions eluted from near the peaks of both the H5 and globin zones in the preparative gel were digested with RNase A and RNase T1 essentially as described by Brownlee *et al.*[20]. Parallel RNase digestion in these conditions rendered *Escherichia coli* rRNA soluble in 5% trichloroacetic acid and did not effect the acid solubility of poly(A). The digested samples were electrophoresed in 10% polyacrylamide gels containing 99% formamide, together with 5S and 4S RNA markers. Two parallel gels included oligo(dT) 17 as an additional marker. The gels were scanned, sliced and the RNA eluted and hybridised with labelled poly(U)[29]. The results (Fig. 3*a*, *b*) showed a peak of hybridisation activity for H5 at a mobility corresponding to 150 ±50 nucleotides and for globin at about 110±40 nucleotides.

The results presented above suggest that a significant proportion of the H5-coding mRNA of chicken red blood cells is polyadenylated.

In the sample analysed, the lengths of the polyadenylate and of the H5 mRNA, together with the protein coding sequence[30], lead to the conclusion that in addition to the poly(A) sequence there are about 260 non-translated nucleotides. The relative amounts of poly(A)$^+$ and poly(A)$^-$ H5 mRNA varied from sample to sample but most of the translatable activity was always found in the polyadenylated material. The binding of H5 mRNA to oligo(dT)-cellulose and poly(U) sepharose is not an artifact due to the high lysine content of H5 (lysine may be coded by AAA or AAG) because, as is clear from Fig. 3*b*, a long fragment of poly(A) is present in the H5 mRNA, and, in addition, short stretches of oligo(A) do not contribute to the poly(U)

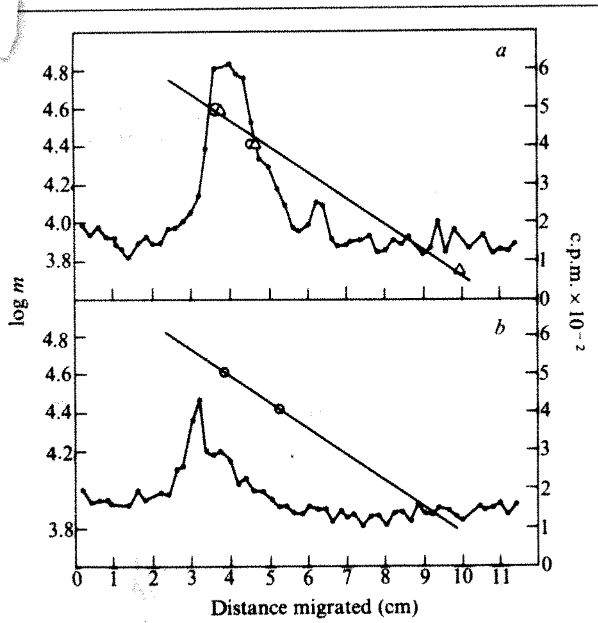

Fig. 3 Poly(A) length measurement. Aliquots of approximately 1 µl of the RNA eluted from slices at 4.5 and 5.4 cm from the origin of the gel shown in Fig. 2*a* were digested with RNase A (RNase to substrate ratio 1:100) and RNase T_1 (RNAse to substrate ratio 1:10) as described[20], in the presence of carrier (0.2 µg) calf liver tRNA. The digests were made 80% in formamide, mixed with 5S *E. coli* rRNA (from Dr V. Erdman) and calf liver tRNA markers and electrophoresed on 10% polyacrylamide, 99% formamide gels made and electrophoresed as described by Pinder *et al.*[27] except that the bis-acrylamide concentration was lowered to 5%. After electrophoresis, the gels were washed, scanned and sliced as described in the legend to Fig. 2. The eluates were analysed by hybridisation with tritiated poly(U)[29]. *a*, Sample from 5.4 cm (globin); *b*, sample from 4.5 cm (H5); △, 5S, 4S RNA, and oligo(dT) 17 markers run on a parallel gel; ○, markers co-electrophoresed. The slope of log *M* versus mobility curve from the two parallel gels was used to draw the lines through the positions to which the markers migrated.

hybridisation. Another possibility is that the H5 mRNA comigrates with significant amounts of polyadenylated mRNA which translates poorly or not at all under the conditions used. Although we cannot rule this possibility out, we consider it to be very unlikely.

The results presented here conflict with the data of Scott & Wells[16] who isolated H5 mRNA from an oligo(dT)-cellulose run-through fraction. They do not, however, make clear whether any H5 mRNA did bind to oligo(dT) nor did they characterise the putative H5 mRNA activity as we have done. We also find, by *in vivo* labelling and from the yields of the specific immunoprecipitated polysomes, that the H5 mRNA activity does not exceed 0.6% of total messenger activity in anaemic red blood cells (manuscript in preparation), a figure much lower than that estimated by Scott & Wells[31].

The translation of H5 in the absence of DNA synthesis suggests that the H5 gene or the mechanisms that regulate its expression differ intrinsically from those for the other histones. The polyadenylation of H5 may be part of the mechanism allowing this histone's unique expression. The genomic location of the H5 gene and the timing of its transcription are among the questions now being examined.

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In vitro simulation of senescence-related membrane damage by ozone-induced lipid peroxidation

K. P. Pauls & J. E. Thompson

Department of Biology, University of Waterloo, Waterloo, Ontario, Canada N2L 3GL

Recent evidence indicates that as cells deteriorate, increasing proportions of the membrane lipids become crystalline (gel phase) at physiological temperature. Microsomal as well as chloroplast membranes from ageing bean (*Phaseolus vulgaris*) cotyledon and leaf tissue acquire regions of gel-phase lipid as senescence advances[1–5]. Gel-phase lipid has also been detected in microsomal membranes from aged batch cultures of green algae (*Scenedesmus quadricauda*)[6], microsomal membranes from regressing corpus luteum of rat[7] and plasma membranes of chick intestinal cells infected with an intracellular coccidial parasite[8]. These observations suggest that the presence of gel-phase lipid in membranes at physiological temperature may be a generalised feature of cell deterioration whether it is attributable to natural ageing or to some kind of infection. Moreover, the resulting mixture of liquid-crystalline-(fluid) and gel-phase lipid in the bilayer is likely to render the membranes leaky[9,10], and could thus readily account for the loss of cellular compartmentalisation that accompanies tissue senescence. The present study reports that phase changes occurring naturally during senescence of bean cotyledons[1,2] can be simulated by exposing isolated membranes from young cotyledon tissue to ozone, a treatment that induces lipid peroxidation[11]. This finding has considerable bearing on previous observations which implicate atmospheric ozone as a source of crop damage by reason of its ability to induce premature plant senescence[12], and lipid peroxidation as a major cause of membrane deterioration in ageing systems[13,14].

Smooth microsomes and cytosol were isolated from cotyledons of 3-day-old etiolated seedlings of *Phaseolus vulgaris* (Kinghorn) grown at 29 °C (refs 15, 16). The isolates were exposed either separately or as a mixture to three 2-min pulses of ozone administered at prescribed intervals over a period of 1 h (Fig. 1). Levels of malondialdehyde (MDA) were measured to determine the degree of lipid peroxidation, and phase changes in the membrane lipid were monitored by wide-angle X-ray diffraction. This technique allows unambiguous detection of gel-phase lipid in membranes and can thus be used to determine liquid-crystalline- to gel-phase transitions[4].

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Figure 1*a* shows ozone concentration fluctuations in the reaction mixture over the 60-min incubation period. These measurements were obtained by bubbling ozone into buffer; corresponding measurements for the full incubation mixture could not be made because the turbidity of the mixture interferes with the spectrophotometric assay for ozone. The buffer was given 2-min pulses of ozone at 0-, 20- and 40-min intervals. Immediately following each pulse the concentration of ozone in the buffer was about 24 p.p.m.; it then decreased over a 20-min period to about 4 p.p.m. (Fig. 1*a*).

Samples of the incubation mixtures were removed for MDA assays immediately after each pulse of ozone. This 3-carbon dialdehyde is produced during oxidation of polyunsaturated fatty acids[17,18], and it is clear that progressively less MDA was produced with each successive exposure to ozone when membrane lipids were the sole source of polyunsaturated fatty acids (Fig. 1*b*, incubation mixture *A*). By contrast, the mixture of membrane and cytosol yielded increasing levels of MDA with each ozone treatment (Fig. 1*b*, incubation mixture *B*). However, treatment of cytosol alone revealed that the extra MDA production could be attributed to peroxidation of cytosol lipids (Fig. 1*b*, incubation mixture *C*). Indeed, when the cytosol values were subtracted, cumulative levels of MDA produced over the 60-min period indicated that membrane treated in the absence of cytosol sustained more oxidative damage than that treated in its presence (Fig. 1*b*).

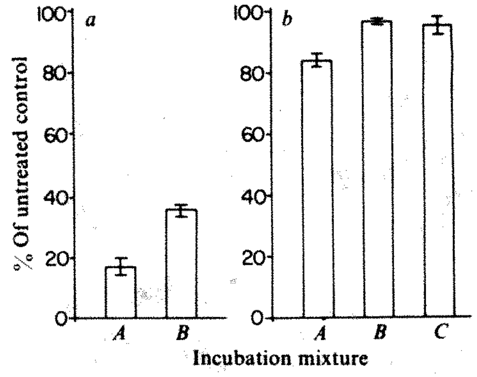

Fig. 2 Levels of phospholipid (*a*) and protein (*b*) remaining in the ozone-treated reaction mixtures after 60 min of incubation. Values are expressed as a % of corresponding controls that were not treated with ozone. Reaction mixtures (the same as described in Fig. 1*b*) were extracted with chloroform/methanol (2 : 1) and levels of organic phosphate in the extracts were determined[34]. Protein in the reaction mixtures was measured by the Lowry method.[35] S.e.m. values are indicated; *n* = 3–6.

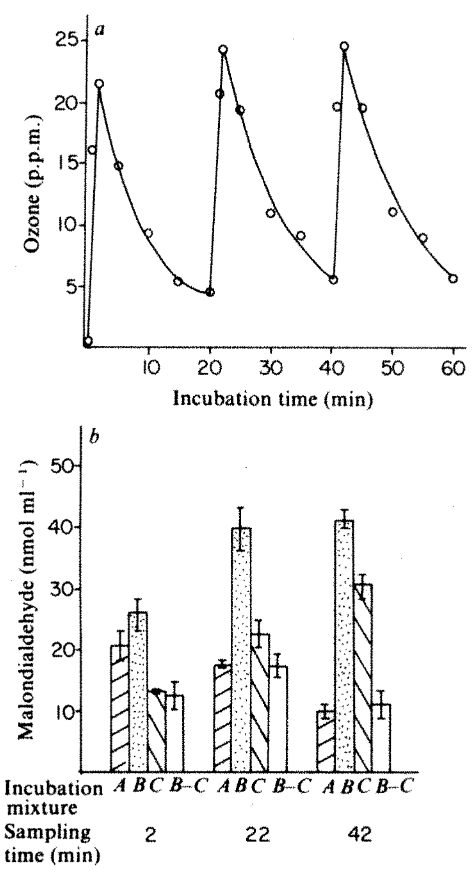

Fig. 1 *a*, Measurements of ozone delivered to the experimental system. Ozone was generated by passing oxygen at 44 ml min^{-1} through an electrical discharge ozoniser, and bubbled with a gas dispersion tube through 30 ml of 0.05 M phosphate buffer, *p*H 7.0, in a 100-ml graduated cylinder placed in a shaking water bath set at 29 °C and 130 c.p.m. A 2-min pulse of ozone was delivered at 0, 20 and 40 min of the incubation period, and the total incubation period was 60 min. Samples of buffer (0.2 ml) were taken at intervals shown and assayed for ozone as described[32]. *b*, Ozone-induced lipid peroxidation. Ozone was delivered as described above to three reaction mixtures containing: *A*, smooth microsomes from 3-day-old cotyledons[15] (0.5 mg protein ml^{-1}) and bovine serum albumin (BSA; 2.5 mg ml^{-1}) in 30 ml of 0.05 M phosphate buffer, *p*H 7.0; *B*, smooth microsomes from 3-day-old cotyledons[15] (0.5 mg protein ml^{-1}) and cytosol from the same tissue[16] (2.5 mg protein ml^{-1}) in the same buffer; *C*, cytosol from 3-day-old cotyledons (2.5 mg protein ml^{-1}) and BSA (0.5 mg ml^{-1}) in the same buffer. All the reaction mixtures and a set of identical control reaction mixtures not exposed to ozone were incubated for 60 min at 29 °C and agitated at 130 c.p.m. Samples (0.2 ml) of each reaction mixture were taken immediately after each 2-min ozone pulse and assayed for malondialdehyde (MDA) using a modified[36,37] thiobarbituric acid test[33]. The values obtained for the experimental reaction mixtures were corrected for levels of MDA found in the corresponding control reaction mixtures. S.e.m. values are shown; *n* = 3.

This was confirmed by measurements of phospholipid at the end of the 60-min incubation period. Incubation mixtures containing membrane and cytosol retained twice as much phospholipid as did those containing membrane alone (Fig. 2*a*). Moreover, lipid seems to sustain much more damage during ozone treatment than protein. For example, the reaction mixture containing membrane in the absence of cytosol retained less than 20% of the original phospholipid after ozone treatment, whereas greater than 80% of the original protein was still present (Fig. 2). The values recorded in Fig. 2*b* are for total protein and do not reflect the relative fates of membrane protein, cytosol protein and serum albumin on ozone treatment, but the proportions of these three sources of protein were very different in the three types of reaction mixture tested, and in each case there was little decrease in protein resulting from the ozone treatment (Fig. 2*b*). In addition, the size of the membrane pellet obtained by centrifuging the incubation mixture containing membrane in the absence of cytosol was consistently smaller than that obtained from the membrane–cytosol mixture, and both these membrane pellets were smaller than those obtained from identical control incubation mixtures that were not exposed to ozone. These observations collectively suggest that cytosol partially protects the membrane from ozone damage by sustaining peroxidation itself. Phospholipid and other organophosphorus compounds were not detectable in the cytosol fractions although lipid (presumably triglycerides) could be seen suspended at the tops of the centrifuge tubes in which cytosol was isolated. This is presumably the source of the MDA produced during exposure of cytosol to ozone.

Wide-angle X-ray diffraction of the membrane pellets from control and experimental incubation mixtures revealed that ozone treatment raises the lipid transition temperature (T_c). As might be expected from the results of the chemical assays, the T_c of membrane treated in the absence of cytosol was raised to a higher temperature than that of membrane treated in its presence. The difference between experimental and control transition temperatures was 18 °C in the former and only 6 °C in the latter (Fig. 3 legend). The diffraction patterns in Fig. 3 demonstrate that the proportion of gel-phase lipid formed is greater in the absence of cytosol than in its presence, and that the amount of gel-phase lipid increases as the ozone treatment is intensified. Indeed, when eight 2-min pulses of ozone rather than three were administered over a 60-min period, virtually all the membrane lipid was in the gel phase at 16 °C (Fig. 3*e*, *g*).

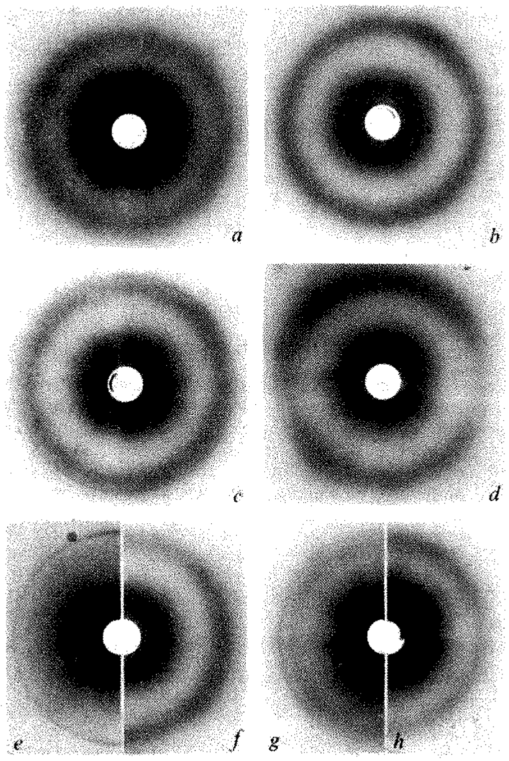

Fig. 3 Wide-angle X-ray diffraction patterns recorded from ozone-treated and untreated smooth microsomes from 3-day-old cotyledons. Reaction mixtures containing membrane and membrane plus cytosol were set up as described for Fig. 1*b*. Control reaction mixtures were given no ozone; one set of experimental reaction mixtures was given three 2-min pulses of ozone at 0, 20, and 40 min, and another set (in 15 ml rather than 30 ml final volume) was given eight 2-min pulses of ozone at 0-min and at 5-min intervals (up to 35 min) thereafter. After 60 min of incubation at 29 °C the membranes were pelleted from the reaction mixtures by centrifugation at 150,000*g* for 1 h. Wide-angle diffraction patterns were recorded from the packed membranes as previously described[1,2]. Patterns recorded at 16 °C for untreated membrane (*a*) and untreated membrane plus cytosol (*b*) show (from outside to inside) a diffuse band at a Bragg spacing of 4.6 Å representing liquid-crystalline lipid and a diffuse band at a Bragg spacing of about 10 Å which probably derives from protein but is not well characterised[1]. Patterns recorded at 16 °C for membrane (*c*) and membrane plus cytosol (*d*) treated with three 2-min pulses of ozone show (from outside to inside) a sharp band at a Bragg spacing of 4.15 Å representing gel phase lipid[1] and a diffuse band at 4.6 Å representing liquid-crystalline lipid[1]. Patterns recorded at 16 °C (*e*) and 55 °C (*f*) for membrane treated with eight 2-min pulses of ozone show (from outside to inside) a sharp band at 4.15 Å in *e* and the diffuse liquid-crystalline band at 4.6 Å in *f*. Patterns recorded at 16 °C (*h*) and 55 °C (*h*) for membrane plus cytosol treated with eight 2-min pulses of ozone show (from outside to inside) a sharp band at 4.15 Å and a diffuse band at 4.6 Å in *g* and only the diffuse band at 4.6 Å in *h*. Transition temperatures (defined as the point at which gel phase lipid first appears as the temperature is lowered) for the experiments illustrated were: *a*, 12 ± 1 °C, $n = 7$; *b*, 15 ± 1 °C, $n = 6$; *c*, 30 ± 2 °C, $n = 5$; *d*, 21 ± 1 °C, $n = 6$; *e*, *f*, 52 °C, $n = 1$; *g*, *h*, 47 °C, $n = 1$.

Moreover, T_c values for membranes receiving this higher ozone dose were 52 °C in the absence of cytosol and 47 °C in its presence. These values for T_c are comparable to those reported previously for the same membranes isolated from extensively senescent 9-day-old cotyledons. Thus, during natural senescence of this tissue, T_c for smooth microsomes rises from 3 °C for 2-day-old cotyledons to 56 °C by day 9 (refs 2, 4, 5).

The specific mechanism by which ozone causes injury to plant tissue is not understood. However, there is considerable evidence that membranes are primary targets. Leaves exposed to ozone display water-soaked spots and become chlorotic and necrotic, symptoms which are thought to reflect loss of membrane selective permeability[12]. Exposure to ozone has been shown to cause leakiness in leaf cells[19], potato tubers[20], unicellular algae[21] and isolated organelles[22,23]. Several observations support the proposal that ozone damage accrues in part from oxidation of unsaturated fatty acids in membranes. Malondialdehyde accumulates[24–26], and there are small decreases in the levels of polyunsaturated fatty acids following exposure of plant tissue to ozone[26]. Lipid peroxidation also renders liposomes leaky[27,28] and increases their viscosity[29]. Our observation that exposure to ozone induces the formation of gel-phase lipid offers an explanation for this increased leakiness, for it has been previously demonstrated that the co-existence of liquid-crystalline- and gel-phase lipid greatly increases the permeability of bilayers[9,10]. The finding that the formation of crystalline lipid during senescence can be simulated by treating membranes from young tissue with ozone supports the contention that lipid peroxidation plays an integral role in senescence, and complements previous observations that anti-senescence compounds such as cytokinins confer resistance to ozone damage on leaves[30] and lower the endogenous level of lipoxygenase[31].

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Erratum

In the letter 'Do sponges help hold coral reefs together?' by J. L. Wulff and L. W. Buss, *Nature* **281,** 474–475, in the footnote to Table 1, in line 6 'does assist with the' should read 'does not provide any'. The last two sentences of the penultimate paragraph should read 'Although the influence of bioerosion should not be underestimated, neither should the binding capacities of other reef sponges. Demosponges clearly require several terms in the reef carbonate balance equation.'

BOOK REVIEWS

Biological oceanography

J.H.S. Blaxter

Developments in Deep-Sea Biology. By N.B. Marshall. Pp. 566. (Blandford: Poole, UK, 1979.) £15.

SEVENTY per cent of the Earth's surface is covered by sea; the *average* depth of the ocean is 3800 metres; its maximum depth is 11,500 metres; its volume is 130 million km^3. These statistics give some idea of size of the last relatively unexplored regions of the Earth, sometimes called "inner space" by the popular press. The science of oceanography (or perhaps oceanology is a better term) started in earnest only 100 years ago during the first Challenger Expedition from 1872—76.

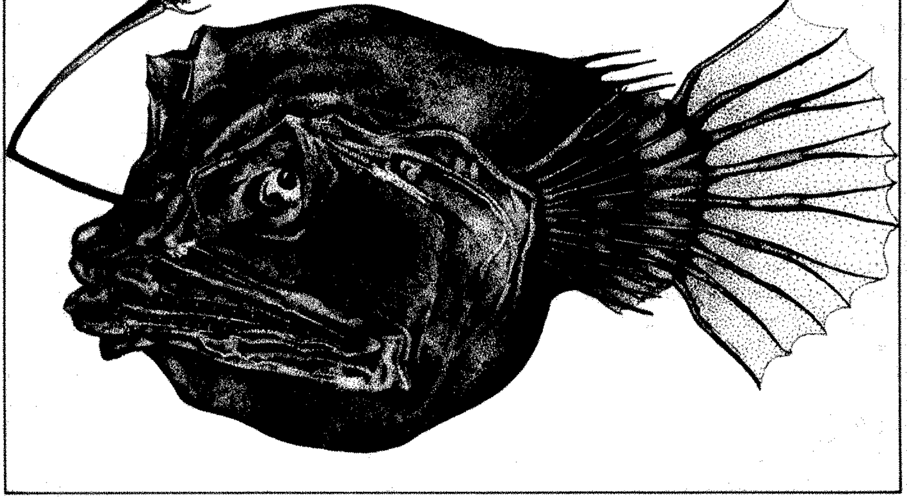
Professor Marshall's book reviews the current level of knowledge of biological oceanography. He attempted in the first instance to revise his 1954 book *Aspects of Deep Sea Biology.* So much had occurred, however, in the past 25 years he set aside his "paste and scissors" (and what a crime it would have been to dissect that volume), and started afresh. The first action of the reviewer was to take the 1954 book from his shelves and compare it with the new one. The latter has 50% more pages which probably indicates an increase of 50% in the volume of knowledge over 25 years. Some of the figures, drawn by Olga Marshall, are fortunately repeated but many are new.

The sections cover Deep Sea Environments; Sea Life, including oceanic plants, pelagic animals and life on the deep sea floor; Deep Sea Food Webs, including those in the pelagic zone, near the deep-sea floor and of the deep sea floor; Physiological Aspects, with chapters on buoyancy, bioluminescence, sensory systems and early life histories; and Biogeography. There is a good bibliography and extensive Species and Subject indexes. There are 170 excellent figures drawn by Olga Marshall and others, and two colour plates by Olga Marshall, the originals of which were hung in the Royal Academy Summer Exhibition.

The book conveys well the esoteric world of the deep sea. The uninitiated will be amazed at the variety and adaptations of oceanic organisms. The initiated will always remember the experience of seeing their first deep sea haul, not only the bizarre forms, but the variety of species and the paucity of the biomass — half a bucket is usually enough to contain the catch from four hours fishing. For every 10 metres depth the pressure increases by one atmosphere; hence many of the animals are living at pressures of hundreds of atmospheres or thousands of pounds to the square inch. Below 1000 metres it is dark except for flashes of bioluminescent light produced by the animals themselves; even above 1000 metres the light is dim and a monochromatic blue. Below the thermocline, which is usually sharp and acts as a barrier both to animal migration and the exchange of water and nutrients, the temperature is an unfluctuating 1—4°C with no seasonal changes. It is the lack of daily and seasonal changes which make the environment so homogeneous. The possibility of lateral distribution without severe physical, chemical or topographical barriers is the reason why deep sea hauls looks so similar, whatever part of the ocean is being fished. The ocean bed, however, is not uniform. Submarine mountain ranges run up the centre of the main oceans; these are the areas where tectonic plates originate which are involved in the separation of the continents. Between the continental shelf and the mid-ocean ridges lie ocean basins, many covered with deep sediments forming abyssal plains.

Professor Marshall describes biological life in the pelagic zone and in the depths. This section of the book is much longer than the previous book, reflecting the advances made since the War. This is essentially a review and in no sense a key to identification. The adaptations can be clearly linked to the shortage of food (large rapacious mouths, recurved teeth, highly distensible stomachs) and the need to attract prey or mate (bioluminescent lures and photophores). The downwardly pointing photophores of some deep sea fish are almost certainly a means for breaking up their form to predators looking up and searching for food in silhouette against the dim downwelling light.

The three chapters on food webs lead to the description of vertical migration, found to be a surprisingly regular and worldwide phenomenon wherever investigations have been made. This rise at dusk to the surface and sinking at dawn towards the sea bed are found in very divergent animal groups and seem to be linked to feeding and the avoidance of competition and predators. Non-migrant related species often occupy separate depth strata, whereas vertical migrants, being able to exploit different depths, often have coincident depth ranges. Although much was known about faunistic variation and the taxonomic make-up of deep sea organisms from earlier investigations, the past 25 years (while bringing some new species to light) has been marked by a better understanding of depth distribution. This is more significant than might be



Female angler-fish (*Oneirodes eschrichtii*)

N.B. Marshall, redrawn from Pietsch, 1974 apparent. It has required great ingenuity to develop equipment to fish at specified depth without "contamination" on the way down and on the way up, before and after the haul. It was disappointing to find that Professor Marshall had not included a section on fishing gear (though he quotes a recent reference) because great advances have been made in this field.

Studies on physiology have also been advanced significantly, although deep sea organisms, especially fish, are difficult to catch in good condition. The physiological discoveries have mainly resulted from a study of functional anatomy and biochemistry, both of which are possible on moribund or even newly-dead animals or on particularly hardy species. A whole range of buoyancy adaptations have been discovered such as low density (for example, ammonical) body fluids, high lipid flotation, reduced skeletons, and special pneumatic float and swimbladder modifications. The sensory systems may be specialised with upwardly directed "telescopic" eyes of high sensitivity with a banked retina of pure rod visual cells. The chemosenses and lateral line may also be elaborated. Life histories have to be maintained on impoverished resources. Often deep sea organisms have no breeding season and produce large eggs which yield larger more viable larvae, and these often rise to the surface during a distributional phase.

Professor Marshall unfortunately says little about oceanic resources, describing the deep ocean as a deserted living space in comparison with the highly productive areas of the continental shelf. Nevertheless, it is true that some of the new species likely to be exploited are further offshore. Apart from lantern fish, which are truly a deep water potential resource, squid, krill and blue whiting are all offshore species found over deep water.

The advances made in biological oceanography since the War must have been expensive: an ocean-going research vessel now costs several thousand pounds to run each day. In the current climate of recession, research vessels may be laid up and it is probable that the next 25 years may not be so productive as the last. Professor Marshall's book has probably been produced at a key time at the end of an almost logarithmic increase in research effort. He is one of the most informed of our deep sea biologists and his book is an important addition to the literature of biological oceanography. It has his characteristic style and it should find a place in the libraries of Universities, Institutes and individuals, as it has the merits of timeliness and readability as well as a modest price. □

J.H.S. Blaxter is Senior Principal Scientific Officer at the Dunstaffnage Marine Laboratory of the Scottish Marine Biological Association, Oban, Argyll, UK.

Ancient climates

W.B. Harland

Climates Throughout Geological Time. By L.A. Frakes. Pp. 304. (Elsevier Scientific: Amsterdam and New York, 1979.) $58.50; Dfl. 120.

SEVERAL books have been published recently on the theme of ancient climates and especially of ice ages. Each has distinctive shortcomings and in every case there is the human limitation that prevents any one person from mastering more than a part of the vast relevant literature. Taking this into account Frakes has without doubt written the best. It is a serious study without journalese or padding and it reflects the author's own work over a wide range of the subject matter. It is a thorough attempt to integrate the latest palaeogeographical reconstructions and, results from the Deep Sea Drilling Program with ^{18}O investigations on palaeotemperatures, by an author with long experience investigating tillites of various ages in more than one continent. Throughout Frakes has carefully considered what is known about the likely properties and motions of the air and water masses enveloping the Earth in relation to the changing pattern of its land and sea. The whole work is internally consistent, having gone through one mind. (In this field too many authors have attempted to support their own prejudices with compilations of undigested data.) A point of view emerges that recurs and has some of the following characteristics.

Frakes gives only 10 out of 267 pages of text to Pleistocene events and he mistakenly includes Pleistocene as a division (the last 1.6 to 2.0 Myr) of the Neogene Period. Such a slip in writing down the Quaternary glaciations would be unthinkable to those brought up in the Northern Hemisphere where earlier ice ages are often treated as an appendix to The Great Ice Age. So this work usefully counter-balances our Arctic preoccupations and brings the southern oceans into play as they developed around Antarctica. There during the last 30 Myr is evidence of ice and a major ice sheet for at least half that time, ice sheets in the northern hemisphere developing only around 3 Myr ago. Although Frakes shows himself to be uncharacteristically ignorant of Pleistocene stratigraphy (and so not able to assess critically the Milankovitch cycles) the reviewer welcomes this refreshing approach that interprets the present as a transient phase evolving out of the past.

Throughout the work there is a continual, at times almost irritating, equivocation over interpretation as the author meticulously discusses each successive problem. So little can be concluded with certainty in considering ancient climates because of the complexity of interacting systems each with negative and positive feedbacks. For example, the interplay of solar constant, ocean gyres, thermal gradients, polar fronts, global albedo, atmospheric CO_2, carbonate compensation depth, biological activity and distribution, ^{18}O, evaporation and precipitation as ice or water, eustasy and tectonics provide the characters in an everchanging choice of scenarios for each episode of history. The first chapter sets out the relevant parameters from atmospheric and oceanic circulation but the applications are repeatedly discussed in context throughout. This explains how it is that many of the questions of palaeoclimatology that were asked more than 100 years ago remain enigmatic.

On the other hand equivocation over tillites and other evidences of ice has virtually been eliminated. The story of ice ages is the skeleton of climatic history and for this Frakes is as well equipped as any from his own researches. There is little new in the account of the broad pattern of glacial history but its integration into the stratigraphical record is thorough. No glacial deposits are recorded older than about 2700 Myr and the reason for supposing the Earth to have been warmer during the preceding 2000 Myr are familiar but well marshalled. A run of well described glacial events between about 2700 and 2100 Myr lack critical age or palaeogeographical control.

Another cluster of very widespread late Precambrian tillites occupies the time span between about 900 and 600 Myr. The receiver especially welcomes Frakes' clear conclusion that the evidence for part of this time, if for no other, requires there to have been low latitude glaciation. He now rules out the prevailing view to which he was earlier sympathetic that the wide distribution of these deposits could be accounted for by the imprint of a wandering pole. He then has to choose between either a global refrigeration that would be difficult to explain without a reduction in the solar constant, or a radically different obliquity at that time (for example, 54° instead of 23.5°). This could be ruled out if high latitude glacial deposits were to be established, for the essence of the obliquity explanation is that with Earth's poles pointing nearer to the Sun only low latitudes could be glaciated.

Phanerozoic history that follows is probably accountable in terms of terrestrial parameters. Palaeozoic ice sheets relate to the southern continent Gondwanaland containing the South Pole and this is a field in which Frakes is a long-standing authority. There may have been ice (possibly continuous) there throughout most of Phanerozoic time (600 to 240 Myr) but the well established ice ages occurred at the end of Ordovician time (435 Myr) and through much of Carboniferous and Permian time (say 330–240 Myr). The highest temperatures have been Devonian (400–350 Myr). The lack of ice in the

Mesozoic Era is fully discussed in terms of oceanic distribution and low albedo leading to peak temperatures in mid-Cretaceous time (say 100 Myr), since when a fluctuating decline ensued.

Frakes' work is scholarly; not every point of opinion is referenced; this may irritate some, but too frequent a punctuation with brackets would have been more irritating. There is, however, an adequate supply of references in the text to most data to be used, with a list at the end of about 500 to 600 works and an adequate index. This book establishes palaeoclimatology as an inseparable part of stratigraphy, itself the documentary basis for understanding most aspects of Earth history. □

W.B. Harland is Reader in Tectonic Geology at the University of Cambridge. He is currently leading the International Geological Correlation Programme Project No. 38 on Pre-Pleistocene Tillites.

Primordial germ cells

C.C. Wylie

Primordial Germ Cells in the Chordates. By P.D. Nieuwkoop and L.A. Sutasurya. Pp.187. (Cambridge University Press: Cambridge, 1979.) £14.50.

THE establishment of the gonad in early vertebrate embryos has fascinated developmental biologists and cell biologists for well over a century now. Basically, the problems fall into the following categories: (1) how is the germ line determined in the early embryo?; (2) what is the mechanism by which the primordial germ cells (PGCs) migrate from the site of their determination to the site of gonad formation?; and (3) what is the mechanism by which PGCs are guided to their target? Thus, many of the cellular processes which make development such a fascinating process are encapsulated in this particular piece of organogenesis, and this has been reflected in the considerable research interest in this subject.

It is therefore timely that Professor P.D. Nieuwkoop's book about the PGCs of the chordates has appeared, for this is a thorough and clear survey of work in this field over the past hundred years. However, despite the authors' considerable contribution to the field, and some five hundred cited pieces of work, we are still only able to answer the above three fundamental questions in the most general way.

Professor Nieuwkoop's main contribution, and indeed, the main preoccupation in his book, is the site and manner of determination of the first germ line cells (the "presumptive" PGCs). The fact that these seem to differ in two vertebrate groups which have been closely studied, the anuran and urodele amphibians, leads him to speculate that the evolutionary tree of the vertebrates, particularly with respect to the amphibians, may be more complex than has been hitherto suspected.

The study of taxonomy through careful study of ontogeny has a long and honourable history, and has tended to be neglected of late. This is largely due to a focussing of research interest on to the cellular and molecular events of embryogenesis, in an attempt to define causative factors in cell differentiation. It is therefore a timely reminder by the author that molecular and cellular phenomena of development are just as important clues in taxonomy as are anatomical features.

This concentration on the taxonomic aspects of the PGCs in chordates is both a strength and a weakness. The weakness is simply because the data on the establishment of the germ line cells, and the route they take to the developing gonad, are still only documented in a fragmentary and unsystematic way. The middle section of the book contains a truly excellent description of this. However, when examined critically, hard evidence on the means by which the germ line is established in chordates only exists for a small number of amphibian species, and not at all for the amniotes, making this a rather tenuous reason for tinkering with the chordate evolutionary tree. The strength of this approach, however, is that it is bound to stimulate research in this subject, particularly into those groups which so far have been neglected. This, in turn, will fulfil Professor Nieuwkoop's aim to gain insight into the way in which the causative factors in early development have themselves changed during vertebrate evolution. □

C.C. Wylie is Senior Lecturer in Structural Biology at St George's Hospital Medical School, London, UK.

Plasma proteins

M.J. Geisow

Plasma Proteins. Edited by B. Blomback and L. Hanson. Pp. 401. (Wiley: Chichester, UK, and New York, 1979.) £22.50.

SWEDISH readers will already know about this book because it first appeared in Swedish in 1976. The latest edition of this multi-author volume is a revised version in English and now includes some literature references up to 1978. The promotors are KABI AB, the Swedish firm which supplies plasma protein fractions.

Plasma Proteins is described as an educational text and a clinical handbook. This seems to be a fair description. The introductory chapter by Kai Pedersen is a small nugget of biochemical history. Apart from exposing the roots of plasma protein science, it also deservedly, but not selfishly, establishes the Swedish contribution to the field. The remaining chapters form a comprehensive account of the biochemistry, pathology and methodology of plasma proteins. The treatment of this material is similar to that in the more expansive work *The Plasma Proteins* (F.W. Putnam, ed., Academic: New York; 1975). In contrast with the latter work, however, you might consider reading the KABI book from cover to cover.

Inevitably for a textbook produced several years ago, there are omissions. For instance, the recent considerable advances in the three-dimensional structure of lipoprotein have not been incorporated in the otherwise excellent description. Nevertheless, as an educational text, the chapters are informative and easy to read. Despite multi-authorship, the closely-linked subjects of defence and repair (that is, the antibody/lymphocyte and blood clotting systems) are treated in a unified fashion.

At a molecular level, there is a pleasing emphasis in this book on the structures of plasma proteins. For example, there are good descriptions of the functions of the transport proteins albumin and haptoglobin in relation to what is known about their molecular structures. A similar molecular structure/biological function synthesis is achieved in the chapters on the protective proteins fibrinogen and plasminogen.

The bench worker is catered for by the thoughtful inclusion of much useful physical and chemical data relating to the individual proteins described. Both historical and current purification techniques are given and there is a discussion of industrial methods for the fractionation of plasma.

At a more clinical level, hospital biochemists have been given a generous amount of space in which normal and pathological states of plasma protein chemistry is covered. There are short but clear descriptions of the main diagnostic plasma protein analytical techniques, with electrophoretic separations in full colour.

I expect to see this book above the benches in the teaching hospitals — both in the pathology labs and in the medical schools. □

M.J. Geisow is a member of the research staff at the National Institute for Medical Research, London, UK.

Invertebrate vision

G. A. Horridge

Handbook of Sensory Physiology. Vol. VII/6: *Comparative Physiology and Evolution of Vision in Invertebrates.* A: *Invertebrate Photoreceptors.* Edited by H. Autrum. Pp. 728. (Springer: Berlin, Heidelberg and New York, 1979.) DM320; $176.

THIS splendid and authoritative series has so far been mainly on vertebrates except where invertebrates illustrate primary processes such as transduction, which was extensively reviewed in Vol. VII, Parts 1 and 2. Comparative studies of photoreceptors by authors who work on insects fill Part 6A but still hardly touch the diversity on invertebrate vision. Final coverage cannot be reviewed because two more sections of Vol. VII, Part 6 are expected.

Despite the emphasis on insects there is a wide spread. The refreshing article by Diehn on photic responses of Protozoa accentuates a need for a large protozoan that can be analysed as a single animal using numerous techniques. The negligible fraction of visual pigment and the small size of *Euglena* really limits advance. The thoughful reviews by Miller of optical filters classified by mechanism and by Snyder on light gathering mechanisms are models of the comparative approach at its best. We almost see the thought processes of the Creator as an optical engineer. Hamdorf's masterly account of visual pigments also gives the impression that so much is now known that we can imagine that most of the diversity is catalogued and the basic physics is understood. For that reason new students easily get the impression from the volume as a whole that the topic is cleaned out, but of course that is not so. For example, the action of any thick lens system such as an eye is understood in terms of principal planes and nodal points, but judging by this volume these are not known for any compound eye: in many eyes even the location of the lens is not known.

The discrepancy between the detailed, almost final, accounts of arthropod visual cells and our relative ignorance about other photoreceptors is emphasised by two splendid reviews: one by Bennett on the entrainment of diurnal rhythms with the conclusion that the receptor is usually not the eyes; and the other, by Yoshida, on receptors for light outside the eyes. It is difficult to understand why one animal group seems to extract the maximum information from its visual system while another seems to rely on a totally ineffective system like a dermal light sense. Why are they not all obviously at the optimum?

The book is, of course, full of in-jokes for the initiated; Stavenga's article doubly so, perhaps from the Dutch. I particularly like his caution that Swihart's "way of reflecting on butterfly eyes must be treated with care" (page 401). Snyder especially recommends an article (1969) by Kirschfeld but omits the reference. The unique index enables one to see at a glance that only two substantial contributors, Steve Shaw and Sigmund Exner, never published with co-workers, and that of the works of Exner, only his book is read today.

The 'classical' works of the past are praised in this volume but sometimes we find a misplaced trust in them and sometimes a neglect of their value. An example of the latter is the "thorough investigation of the butterfly visual sense cells" quoted by Stavenga. By measuring the reflection Bernard "made the startling discovery of a red absorbing visual pigment of which the spectrum peaks at about 610 mm". If he had read Max Schultze's classical paper on the compound eye written in 1868 Bernard would have related his red reflections at least partly to the strings of red pigment in the retinula cells. We cannot, however, always believe the classical authors. For example, Exner interpreted these red strings as additional out-of-focus rhabdoms. This was all noted by Ribi in a paper which Stavenga actually quotes in his references but does not incorporate. In fact, in every corner of the subject, as in all science, there are not only great gaps in our knowledge awaiting future genius, there are plenty of murky corners to be cleared out, also requiring lucidity and experiment. One notes the pietism on page 417: "One may assume from the present high interest in photoreceptor physiology and the accordingly rapid accumulation of new data, that some crucial questions will soon be answered"; but interest and data are not the whole story. □

G.A. Horridge is Professor of Neurobiology in the Research School of Biological Sciences, Australian National University, Canberra.

Silica chemistry

D. Dollimore

The Chemistry of Silica: Solubility, Polymerisation, Colloid and Surface Properties, and Biochemistry. By Ralph K. Iler. Pp.866. (Wiley: New York and Chichester, UK, 1979.) £39.50.

RALPH ILER is well known for his research publications and for his earlier book. *The Colloid Chemistry of Silica and Silicates* (Cornell University Press: Ithaca, New York, 1955). This new book will be judged by a comparison with the old. It must be stated that the author first intended this new venture to be an updated second edition of the earlier book. It must, however, in this expanded form be regarded as a new work.

The purpose of the present book is to provide an account of the chemistry of silica, including soluble silica and silicate precursors of soluble silica, the polymerisation to polysilicic acids, colloidal sols and gels, and the surface chemistry of silica. This provides the reason for the provision of the subtitles shown above. The consideration of some 2,500 new references since the first book make the comment of the author that science students graduate with little knowledge of silica's properties or chemistry very pertinent. It may be that this book will be an incentive for the provision of more silica chemistry in the undergraduate syllabus. The author states that the book is written for those engaged in work on silica, as a guide to the literature and for those in other fields who need specific information on the subject. The author does this in a book of 866 pages.

In a short introduction Dr Iler describes previous books and reviews of silica chemistry. He also defines terms such as Silanol, polymerisation and silica. Other names are, however, explained at the point of introduction in the text.

The subject is introduced by the consideration of the solubility of different forms of silica, the factors that influence dissolution and deposition, and the other aspects of the solution chemistry of silica. The various analytical methods are described and compared, and recommended methods given in detail.

Water-soluble silicates are then described as a prelude to the chapter on the polymerisation of silicic acid. A silica sol formation mechanism is clearly detailed together with the necessary factors that control the properties of the system. The various types of gels and powders are characterised, with a great deal of attention paid to their adsorption properties and their uses. There is a chapter on the role of silica in biology, in which the aspects of the occurrence of silica (including toxicity) in mammals and other life forms are discussed. □

D. Dollimore is Reader in Physical Chemistry at the University of Salford, UK.

nature

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Debugging systematic bacteriology

THE International Committee on Systematic Bacteriology (ICSB) has reason to be pleased with itself. For there is shortly to appear in the *International Journal of Systematic Bacteriology* the ICSB's new Approved List of Bacterial Names, a list that will include only about 2500 of the 30,000 bacterial species that have been named over the years. Only a handful of those species that have been excluded are likely to worm their way back onto the approved list since, by the standards laid down by the ICSB, their alleged identity is unacceptable.

The identification of bacteria dates back, in principle, to 1753 and the *Species Plantarum* of Linnaeus although in practise it was only from the middle of the nineteenth century that identification of species started in earnest. The problem that has grown ever since then is that by modern standards the vast majority of named species were given descriptions that were totally inadequate for them ever to be recognised again. Furthermore, for very few of the 30,000 alleged species was there a type culture — that is, an authentic specimen of the original beast — with which any new specimen could be compared.

In these circumstances it had become almost impossible for anyone to identify, unambiguously, a new species of bacterium. To do so meant laboriously consulting old and inadequate literature. It meant sifting through hundreds of names, scores of which might apply to the same species. Experts would disagree about which names applied to the same species and which single name to opt for. The competing names often lingered on in the literature with each in turn being overturned or revived as new evidence turned up. And when the issue might have been settled by re-examining the species themselves, cultures either did not exist, were of dubious authenticity or had clearly become outgrown by contaminating bacteria.

It was because of this situation that the International Committee on Systematic Bacteriology of the International Association of Microbiological Societies was persuaded to undertake the formidable task of hacking away the dying, decaying and merely dubious foliage in the hopeless jungle of bacterial nomenclature. After years of committee work, the experts have decided that there are only about 2500 species of bacteria that are currently recognised by most bacteriologists and which possess type strains or equivalent type material. From now on bacteriologists need only consider these approved species and, of course, any that are subsequently added to it. Additions will be carefully controlled by the requirement for better descriptions and designations of type strains than in the past. More controversially, new names will only be acceptable once they have been published in the *International Journal of Systematic Bacteriology*

Had the last requirement amounted to a monopoly of publication there would have been room to criticise it both on principle and as a workable proposition. The committee, however, have acknowledged that publication of the full details of newly named bacteria can be in any scientific journal or book whilst stating that they will not validate any name until it has been announced (with reference to the full paper) in the *International Journal of Systematic Bacteriology*. This makes good sense since it will mean that any initial search through past names can henceforth be made in a single journal.

It is also good that the Judicial Commission of the ICSB is willing to consider proposals to re-admit bacteria that have been excluded from the approved list. No gathering of experts, however conscientious, could have hoped to make all the correct decisions and doubtless a small number of old names will get back on the list. The Judicial Commission should be firm but sympathetic to anyone who is willing to champion the re-appraisal of their pet species provided that they are prepared to produce adequate evidence, a modern description and a satisfactory type strain.

This attempt of bacteriologists to put their house in order has implications for other biologists. The great conservatism of nomenclaturalists is well known. The idea of abandoning the principles of priority is anathema to them. But when the application of the principle causes more instability than the stability it is intended to preserve, then it is indeed time to make a fresh start. The plan adopted for bacteria may be directly applicable to only a few other groups of living organisms. But the principles followed in the plan may be of wider use when the nomenclature of other groups of living creatures, or of other scientific entities (eg genes, enzymes, ecosystems) comes to need revision. □

New UK Select Committee needs more attack

ONE of the first trials of the new UK select committee system took place last week, when the House of Commons Select Committee on Energy confronted the Secretary of State for Energy, Mr David Howell, and his junior ministers, on the government's recently announced nuclear policy. The Secretary of State undoubtedly won the battle.

Not that that was entirely due to the integrity of Mr Howell's case. While Mr Howell is a thoughtful minister, and well-briefed by his large department, there are inevitably weak points in his thinking and lacunae in his policy; and it is the job of the Select Committee to bring them out.

For example, what of the accusation that if the £10-12 billion of government money allocated to the nuclear plan had instead been allocated to existing and proven conservation technologies, three times the primary energy value of the reactors could have been saved? On a recent television programme, Mr Norman Lamot, the minister responsible for nuclear affairs, answered this with a remark about it being impossible to control the needs and desires of energy consumers, missing the point completely that both the nuclear plan and a conservation plan are matters of direct government spending, as when the gas-cookers of Britain were converted, at government expense, to North Sea gas.

Further, it is clear that one of the principle objectives of ordering ten new nuclear reactors is to assure the strength of the Biritish nuclear industry. But after the long history of set-back with the advanced gas-cooled reactor, and the loss of many highly-trained staff, is the industry capable of carrying the orders out?

The new arrangement of select committees, which parallels the arrangement of government departments and has hence eliminated the useful form of the Select Committee on Science and Technology, was intended to give MPs a more direct basis of attack on government policy. But on last week's performance it seems unlikely to have that effect. More of those serving on the committees should be prepared to do some hard homework, and the committees should be more prepared to organise themselves to attack and expose the weak points in government thinking. Neither the homework (except in one notable case) nor the organisation were particularly evident at the Select Committee on Energy last week. □

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OSHA looks at occupational regulations for recombinant DNA use

REFLECTING the increasingly rapid movement of recombinant DNA techniques from the research laboratory to the industrial production line, occupational health agencies in Washington are beginning to discuss how they should handle any health hazards that may be involved.

Dr Eula Bingham, Assistant Secretary of Labour and head of the Occupational Safety and Health Administration (OSHA), has suggested that an expert panel be set up to address the hazards of DNA use "particularly as they apply to potential occupational exposures".

In a letter to Dr Donald Fredrickson, Director of the National Institutes of Health, Dr Bingham says she supports the system of voluntary compliance by private industry with the NIH guidelines which, after a period of public comment, was implemented last week.

But she adds that the agency feels that "more formalised enforcement of procedures and rules within the regulated private sector is warranted" — a feeling shared with Senator Adlai Stevenson Jr who last week introduced a bill into the US Senate requiring mandatory registration of all appropriate experiments not currently covered by the guidelines.

Dr Bingham has suggested that the new panel should be established under the chairmanship of Dr David Rall, Director of the National Institute of Environmental Health Sciences — and that it should include not only members of the NIH's Recombinant DNA Advisory Committee, but also technical representatives from unions, management and public interest groups.

She also suggests that OSHA might review the NIH guidelines and publish them as interim guidelines for industry. "While these do not hold the force of regulations, they would indicate our support for control in this area and would also produce information to employees indicating what we feel are appropriate precautions and procedures of recombinant DNA use".

Dr Anthony Robbins, Director of the National Institute of Occupational Safety and Health (NIOSH) told *Nature* this week that he agreed with OSHA that the time was appropriate for detailed consideration of the implications of occupational exposures. Commenting on the fact that, in the past, OSHA has been reluctant to involve itself in the debate over the safety of occupational activities involving recombinant DNA techniques, Dr Robbins said that previously the agency had hoped this would be done elsewhere. "Since no one else seems to be doing this, it seems to be the time to move", he said.

NIOSH is, in particular, suggesting that a registry be set up of all workers involved in such actitivies to enable the long term health effects to be adequately monitored. And as a precedent, he points to the recent decision by the Swedish government to set up a commission on the occupational hazards chaired by the head of the National Labour Protection Board.

No response to Dr Bingham's proposals has yet been formally received from Dr Fredrickson, who last week issued virtually unchanged revisions to the NIH guidelines which he had proposed last November and which significantly reduce containment requirements for a wide range of experiments.

In particular, Dr Frederickson has implemented his proposals that most experiments involving the bacterium *Escherischia coli* K-12 be reduced to P1 plus EK1 containment levels, and that such experiments no longer need be registered with the NIH.

In announcing his decision, Dr Fredrickson said that he had received five letters of comment suggesting that the relaxation of the guideline was premature. However he had also received more than 170 letters giving general approval to the proposed changes, which he had decided to implement as originally suggested.

Dr Fredrickson's announcement, widely welcomed in the scientific community, coincided with the introduction of the bill tightening up the requirements for private industry introduced by Senator Stevenson, chairman of the Senate Subcommittee on Science and Space. Explaining the general purpose of his bill, Senator Stevenson said that in a time of general concern about the health of industrial innovation, the conditions surrounding recombinant DNA research seemed "unusually favourable" for the achievement of significant innovation — and that in the circumstances government regulations should not be undertaken lightly.

"However the modest registration requirement I propose is justified as a minimal precaution against inadvertent harm to public health or the environment. Far from impeding the orderly progress of the research and its commercial exploitation, mandatory registration may actually facilitate it." ☐

Earth sciences and remote sensing agreements signed with China

THE United States and the People's Republic of China last month signed two protocols for scientific and technological cooperation in earthquake studies and earth sciences, as well as a memorandum of understanding on the use of the Landsat earth resources monitoring satellite.

The signing of these accords came almost exactly a year after President Carter and Vice-Premier Deng Xiaoping signed a general agreement on scientific and technological cooperation between the two countries in Beijing (Peking).

The earthquake studies protocol includes cooperation in earthquake prediction, earthquake hazards evaluation and earthquake engineering, as well as other related basic and applied studies. Joint research in these areas is to begin immediately.

The other protocol covers research in areas such as marine resources, energy resources, marine geology and geotectonics. The two protocols cover various forms of cooperation between scientists in the two countries, including exchange visits, joint organisation of scientific conferences, symposia and lectures, and the exchange of specimens and standard samples.

Under a separate agreement with the National Aeronautics and Space Administration the People's Republic will purchase a ground station to receive, process, archive and disseminate earth resource data from the Landsat D satellite.

The station will be located near Beijing and the Chinese Academy of Sciences has agreed to help share in the cost of operating the satellite by paying an annual access fee of $200,000, beginning six months after the station starts to receive data.

Other areas agreed by the Commission for possible future cooperation included aviation, statistics, nuclear energy (including nuclear physics and nuclear fusion), electronics and telecommunications. Administration officials in Washington last week rejected suggestions that moves to improve scientific and technological links with the People's Republic of China were directly related to the recent cooling of political and scientific relations with the Soviet Union.

Appearing before a Congressional committee hearing, Dr Frank Press, Director of the Office of Science and Technology Policy, said that scientific exchanges with China had been a positive element in the process of normalising US-China relations. **David Dickson**

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Andrei Sakharov

US cuts back on official exchanges with USSR

THE US scientific community has been almost unanimous in condemning recent moves by the government of the USSR, in the occupation of Afghanistan and the internal exile of physicist Andrei Sakharov.

But while many support the legitimacy of individual protest, there remains uncertainty over how far official reaction should undermine the network of bilateral exchange agreements which have, in recent years, begun to produce mutual benefits.

On the one hand, Congressman George Brown, last week introduced a bill calling for a one-year moratorium on US-Soviet exchange except those essential to national needs or a matter of extraordinary circumstance of individual conscience.

The Carter administration, however, while keen to make clear to the Soviet government that it is not 'business as usual' for scientific exchanges, is also concerned to maintain what it can of the existing framework for scientific cooperation.

Thus all high-level meetings between scientists and scientific administrators from the two sides are being deferred. Three such meetings have already been postponed since the occupation of Afghanistan, and all current exchange arrangements are undergoing close review.

But the administration is at present keeping the door open for low-level contacts between scientists which it considers to have a purely scientific or humanitarian purpose. For example, a scientific delegation from the USSR studying the biological control of pests has been allowed in — but a delegation coming from the USSR to discuss science policy has not, since it was lead by a deputy minister.

The administration's determination to keep open channels of communication, even if limiting activities in these channels, was supported by Academy President Dr Philip Handler, who will be leading the US delegation to the scientific forum in Hamburg later this month to discuss the progress of the Helsinki accords of 1975. But he emphasised the Academy's position that the main responsibility lay on the shoulders of individual scientists.

Uncertainty over how tough the administration should be is reflected in the debate over what type of strategy the US delegation should pursue at the Hamburg science forum.

Few currently support a complete boycott of the meeting. And there seems general agreement that human righs considerations are central to international scientific communication and exchange.

But given agreement that the forum should discuss, in Dr Press's words, "the context within which scientific cooperation takes place, not simply those scientific subjects which are amply discussed in many other settings", opinions are divided on what such discussions should aim.

Dr Paul J Flory, for example, Emeritus Professor of Chemistry at Stanford University and a member of the US delegation, suggested that the scientific community should "reshape its criteria for participation" and lay down a set of minimum conditions for future international collaboration.

These might include the condition that "negotiations should be in the hands of scientists, not governments", and that participants be selected "without regard for their political conformity, race or ethnic background".

Others have suggested more modest goals. In particular Dr John T Edsall of Harvard University, chairman of the American Association for the Advancement of Sciences' Committee on Scientific Freedom and Responsibility, has suggested that the forum should establish a working group to collect and review reports about obstacles to international scientific cooperation. **David Dickson**

Soviet Union responds to western reaction

WITHIN a few minutes of the news of Sakharov's exile reaching the West, the Royal Society sent a cautious telegram to the Soviet Academy of Sciences, deploring the action and asking for the Academy's comment. Six days later, the Academy made its opinion known in a formal statement by the Presidium.

The statement condemned the activities of Academician Sakharov as being directed "against the interests of our country and the Soviet people, actions that help to build up international tension and bring into disrepute the exalted title of Soviet scientist". Ironically, the Presidium's statement accuses Sakharov of "undermining" precisely those ideals of peace, arms limitation and detente that underlie his whole involvement with human rights. Last year, indeed, he spoke out resolutely against overenthusiastic efforts to link US willingness to sign SALT-2 with human rights in the USSR.

Nevertheless, the statement contained no indications that moves would be made to expel Sakharov from the Academy. While feelings of solidarity may so far have prevented Sakharov's fellow-Academicians from expelling him from their ranks, there is little doubt that scientists of dissident outlook and lesser prestige now feel themselves under increasing threat. Andrei Tverdokhlebov, the young physicist, who had formerly worked closely with Sakharov in the human rights movement was expelled to the West just in time to testify before last week's congressional hearing in preparation for the Hamburg scientific forum. And a number of Jewish refusniks, notably Naum Meiman the mathematician and Aleksandr Lerner the cybernetician, have expressed fears for their own liberty.

Meanwhile, the Soviet authorities are attempting to counter western concern by denying that Sakharov has been, in any sense, exiled. "Administrative banishment", said one Tass statement "is neither 'arrest' nor 'exile'." Gor'kii, said comentator Yaroslav Khabarov on the foreign service of Moscow radio, "is one of the most beautiful towns in Russia, as well as a major industrial, cultural, and scientific centre with its own university". By simply stripping Sakharov of his awards and titles, and "moving him outside the city of Moscow", Khabarov explained, Sakharov has been given "the opportunity to continue to work in keeping with his profession". **Vera Rich**

USA
MAYBE NOW I'LL GET AN EXCHANGE TO THE USSR!
VICE DEPUTY CHIEF EXECUTIVE DIRECTOR JONES
CCCP
MAYBE NOW I'LL GET AN EXCHANGE TO THE USA!
VICE DEPUTY CHIEF EXECUTIVE DIRECTOR IVANOV
Tony Johnston

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Soviet Union

New appointment binds science closer to industry

ACADEMICIAN Gurii Marchuk has been appointed Chairman of the State Committee for Science and Technology, in place of Academician Vladimir Kirillin, whose resignation, was announced on the same day as the banishment of Andrei Sakharov from Moscow.

The appointment of Kirillin's sucessor came remarkably quickly. The post is of ministerial rank (and carries with it, virtually automatically, the position of a Deputy Chairman of the Council of Ministers). Posts of this calibre are seldom filled immediately — a lapse of several months has, of recent years, been the rule. Moreover, at 54, Marchuk is unusually young by Soviet standards, for high office. The average age of the country's leadership is in the late 60s.

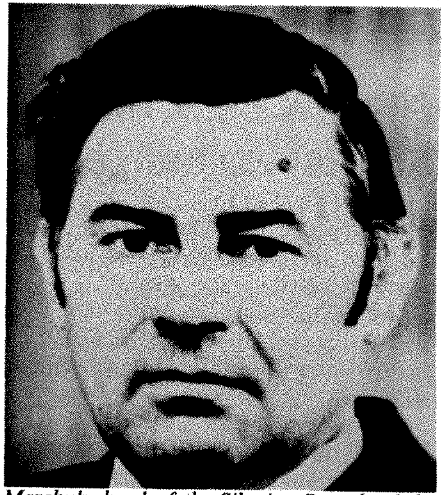
Not that Marchuk is unknown to Soviet science. He has already held the corresponding position of head of the Commission for Science and Technology of the Soviet of Nationalities (lower house). More important, he is head of the Siberian Branch of the Academy of Sciences.

The Siberian Branch of the Academy was set up in 1957, housed in the new Akademgorodok (Academic Village) at Novosibirsk, and briefed specifically to work on scientific problems of greatest relevance to the development of Siberia and the far east. By 1966, the branch had become surrounded by a network of research institutes, design bureaux and experimental production factories subordinated to individual ministries but under the scientific supervision of the branch.

This system of dual control has caused concern to a number of Soviet scientists, who have felt that the ministries might burden the R&D facilities of the branch with small-scale, day-to-day tasks, or even bypass the scientists altogether in decision-making.

Since he took over the chairmanship of the branch in 1975, Marchuk has written extensively on the dual control problem. Although at times he has complained that certain ministries have been lax in providing suitable conditions for successful work, his overall view is that the dual control system works well.

Of recent years, there has been constant emphasis in the Soviet press that science and industry must be more closely linked.



Marchuk: head of the Siberian Branch of the Academy of Sciences

Last November, when castigating the State Committee for slackness, Mr Brezhnev suggested that the Academy of Sciences should take a greater lead in organising the applied research the country needs. The precise division of labour and responsibility between the State Committee and the Academy is a complex subject (the head of the State Committee is regularly a Vice President of the Academy). Marchuk's Novosibirsk background, however, suggests that the long-discussed Soviet policy of integrated planning and the close coordination of research and production will be a keynote of the next five year plan for science.

Vera Rich

United Kingdom

Industrialist made part-time director of biophysics unit

THE UK Medical Research Council (MRC) has taken the unprecedented step of appointing as a part-time director of one of their research units a scientist who is permanently employed within industry. As soon as Professor Maurice HF Wilkins retires as Honorary Director of the MRC Cell Biophysics Unit at Kings College, London, he will be replaced by the combination of Dr David Rees, 43 year old Executive for Scientific Policy for Unilever Research and 56 year old Professor Brian Boycott who is on the Unit's staff. Whereas Professor Boycott's post will be full time, Dr Rees will be in the service (and pay) of the MRC for only one day per week, on average, and will retain his present position with Unilever.

The hunt for a new director has been on for well over a year since Professor Wilkins let it be known that he would like to retire as soon as a replacement could be found. But it has proved extremely difficult, and ultimately impossible, to attract someone of the right calibre and scientific background to be director. The solution adopted is based on the belief that Dr Rees' scientific credentials as a carbohydrate chemist with a strong interest in cell biology will complement those of Professor Boycott whose expertise is as a neuroanatomist of the retina.

Professor Boycott told *Nature* that he was glad to have someone familiar with current molecular biology as an Associate Director. The MRC expects Dr Rees "to inject good science into the unit" first by spending a continuous two to three months there and then by one day a week appearances and through having two of his research staff working in the unit. In return they hope that members of the unit will wish to sample the facilities and atmosphere of the Unilever Research laboratories.

A spokesperson for the MRC said that the link with industry was an experiment as much of serendipity as of design but that the MRC was now anxious to foster relationships between itself and industry. Although no other links were currently planned, as opportunities arose they would be considered seriously. □

Temperate vegetable seed bank set up

OXFAM, the Oxford-based famine relief organisation, has raised £700,000 by special appeal to establish a seed-bank for vegetables. A target of some 10,000 species will be collected in a -20°C store associated with potting facilities, laboratories, and greenhouses.

According to Oxfam's director, Mr Brian Walker, two-thirds of the seeds collected will be temperate, and only one-third tropical; but, says Walker, "in many parts of the Third World people eat temperate vegetables: cauliflower, onions, carrots, potatoes, cucumber, tomatoes.

"In a tropical country you only have to go a little way into the hills to meet temperate conditions. A large part of India's potatoes are grown in the hills." Moreover, winter in the northern plains of India was little different from the growing season in the US.

The origins of the Oxfam initiative go back to the Rome food conference of 1974, when it gathered a group of senior scientific advisors to survey world seed-banks and report on deficiencies. The group reported that while there was good coverage of cereals, there was no embracing collection of vegetables, which are important particularly for people growing cash crops around the borders of towns.

The seed-bank will be established at the UK Agricultural Research Council's Vegetable Research Station at Wellesbourne, Warwickshire. Oxfam's £700,000 will pay the capital costs, and the running costs for the first seven years. Then the bank will be taken over by the ARC. □

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NEWS IN BRIEF

Stanford president to head Rockefeller Foundation

Dr Richard W Lyman, currently President of Stanford University, California, has been chosen from a field of 1000 nominees to head the Rockefeller Foundation. Dr Lyman has been President of Stanford since 1970, and will leave his post in the summer. He will succeed Dr John H Knowles, who died last March, as President of the Foundation, of which he has been a trustee since 1976. University officials at Stanford will meet next month to discuss the selection of a new president.

US airlines told to restrict ozone intake

TEN years ago, it was a question of whether high flying aircraft would cause permanent damage to the Earth's ozone layer. Now US scientists are concerned that an excess of ozone inside such aircraft could be a hazard to passengers. And the US Federal Aviation Administration has issued rules requiring aircraft to reduce the amount of ozone entering aircraft, based on standards established by the Occupational Safety and Health Administration.

Initially proposed in 1978, the new rules will require airlines to screen out most ozone as it enters airline cabins by means of charcoal filters — or to follow routes that avoid areas of high ozone concentrations. On flights of less than four hours the maximum concentration of ozone will be 0.25 parts per million. On longer flights, the average level must be no more than 0.1 parts per million.

Pugwash takes up East-West dialogue

THE informal diplomacy practiced for 25 years by the Pugwash conferences was brought into play on 19-20 January in Geneva to "take up the slack caused by the present suspension of official East-West dialogues". Convened by the Pugwash Executive Committee, thirty three participants from 19 countries including high ranking Soviet and NATO officials met to discuss "the critical nature of current developments and the increasing danger of nuclear confrontation". Described as "useful", the talks included exchanges on the subjects of Afghanistan and the destabilising effects of the NATO nuclear modernisation programme. The participants will continue discussions at another specially covened meeting to be held in mid-April.

In the meantime the British Council of Churches is continuing its campaign against the installation of the latest Polaris missiles on UK submarines (29 November page 435). BCC representatives met with the Ministry of Defence last week where they raised the question of the military relevance of a UK submarine nuclear threat that would be under NATO, not UK,control. Citing the opinions of Lords Mountbatten and Carver in support,BCC representatives cautioned the Ministry against renewing the Polaris as "a matter of political machismo".

PETRA to boost luminosity by ten

PETRA, the existing 19 GeV on 19 GeV electron storage ring at DESY, Hamburg, will install the last of 60 accelerating cavities in February and so for the first time reach its peak energy. And as the luminosity of storage rings increases rapidly with energy, it is hoped to raise the present 50 per day event rate by a factor of ten. This should make it possible at last to perform the 'forward-backward asymmetry' experiments which should detect the low energy 'tail' of the Z^0, and bring early indications of the presence of the particle. According to the Weinberg/Salam unified theory of the weak and electromagnetic interactions, the Z^0 (intermediate vector boson) plays the same role in the weak interaction as the photon in the electromagnetic interaction. At 90 GeV, it would be the heaviest particle yet found.

Basques blow up nuclear power plant equipment

MEMBERS of the Basque separatist organisation ETA blew up electrical equipment scheduled for delivery to the troubled Lemoniz power plant near Bilbao last Sunday. A group of armed men captured the factory manager of the Segasa factory in Vittoria at his home, took him to the factory where they disarmed eight guards of their .38 calibre pistols and placed plastic explosive around special batteries intended for the Lemoniz plant. Warning the guards and manager to stay clear of the blast, the men left and the batteries exploded a few minutes later. The action occurred during a weekend of revolutionary violence in which ten people were killed in armed actions throughout the country.

Denmark shelves nuclear power indefinitely

THE Danish government last week announced an indefinite postonement of the introduction of nuclear energy. In a major policy shift, said to be strongly influenced by the accident at Three Mile Island, the ruling Social-Liberals supported by the Single Tax party, two smaller left wing parties and the major trade unions — who had previously supported nuclear power — decided to defer any parliamentary action on the question.

Citing uncertainties about safety and waste storage, Energy Minister Poul Nielsen pointed out that Denmark was too small to support safely more than five nuclear plants which would only supply 15% of Denmark's energy needs. In its place, development of North Sea gas in conjunction with Norway is expected to cover the major part of Danish, Swedish and Norwegian energy needs well into the next century with enough left over to export to West Germany. The government will now draft a new energy plan that will emphasise, a shift from oil to gas, energy conservation and increased commitment to the development of alternative energy sources.

French researchers protest CNRS reorganisation

FRENCH scientists of the Centre National de Recherche Scientifique continued their opposition to management reorganisation plans by holding a one day strike and demonstration last week. More than 1500 research scientists marched through Paris to the offices of the Secretary of State for Research to protest against recent changes in the law which would introduce a new four year probationary period, lower the maximum age for entry to research to 27 years and permit forced mobility at the discretion of research directors. The trade unions representing the researchers sent an open letter to Prime Minister Raymond Barre signed by 1000 scientists including 300 research directors, senior researchers and professors that demanded a suspension of the decrees as measures that "would compromise gravely the future of basic research in France".

SRC clamps down on post-docs

THE UK Science Research Council has instructed its research directors to apply "rigidly" all rules concerning employment of post doctoral research fellows. The orders, contained in a memorandum circulated to SRC research establishments last November will effectively prevent research scientists on post doctoral contracts from gaining extensions or further contract work when their present contracts expire. The SRC defends its policy in ethical terms. Stating that the reasons for the policy were not financial — "very few people will be affected" — an SRC official said "we have a moral responsibility to encourage new ideas. People who have been here six years have had their chance and now they must make way for fresh blood".

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FEATURES

Research centres, libraries, journals flourish in new China

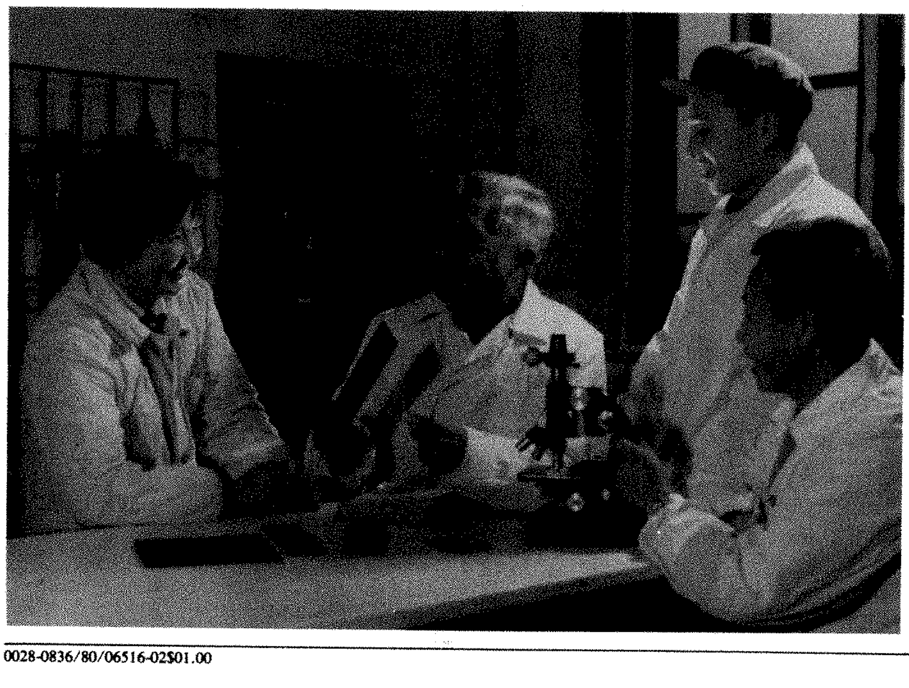
In the second of three articles on the development of science and technology in China, **Tong B Tang**, research fellow at Darwin College, Cambridge, UK, who visited China at the end of last year, examines scientific services

MOST Chinese scientists — indeed, the people as a whole — are friendly, forthcoming and purposeful, yet modest and earnest (sometimes to the point that reserved westerners find uncomfortable to reciprocate). Their optimism is derived from the positive and negative lessons of the Cultural Revolution (reputedly assessed by Chairman Mao to be 70% and 30% respectively), open discussions on present problems, and from a common resolve to achieve the four modernisations in agriculture, industry, national defence, and science and technology. To this aim, the political decision-making system is being scrutinised and modified to prevent the recurrence of a dictatorship by a minority, like that of the Gang of Four (GoF).

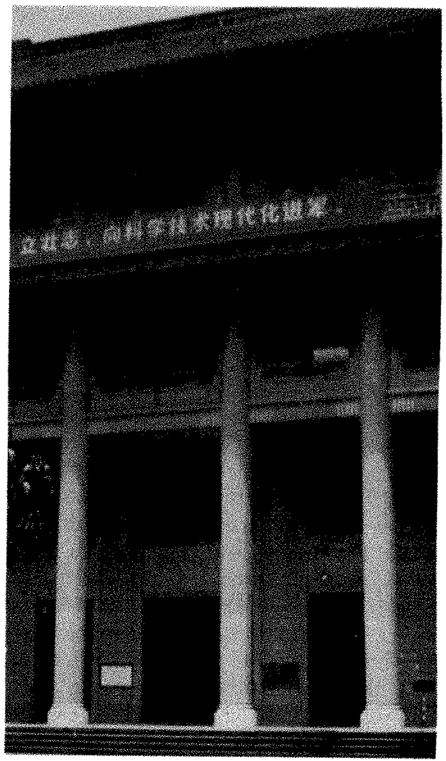
Both science and scientists suffered under the GoF. This was probably due not so much to deliberate policies as to the GoF's broadside attacks on "revisionism" and on people it labelled as "capitalist-roaders". Although the GoF had some supporters its treachery became increasingly clear with time: its attacks constituted the means rather than the end of its attempt to seize power. Sectarianism resulted and this reached a peak in 1975 when Mao condemned the factions semi-publicly. The aim of the present leadership is an extended period of stability and economic construction. The conditions are right for the growth of science.

Not surprisingly the Scientific and Technical Association, which barely existed three years ago, is expanding. Often affectionately referred to as the 'home' of scientists, it sponsored some 150 meetings in 1978; the number last year exceeded 650. The newest members include a futurology society and a solar energy society. As well as its function as a learned society, the association is responsible for the popularisation of science, which I shall talk about next week. Neither it nor any of its member societies acts as a qualifying body.

After the reopening of their 'home', our Chinese colleagues are also benefiting from other improvements. Problems had included insufficient time for research owing to a shortage of assistants and too many administrative commitments and family inconvenience such as wife and husband having to work in different institutions in different places. Now that research and/or teaching are judged to be the scientists' main duty, conditions of work are guaranteed. The buildings and appliances such as power points are often old and spartan by western standards, but functional. In general there are still not enough instruments yet, reassuringly, for the most part they are designed and produced in China. Laboratories may look run-down, but everyone using them knows exactly how the equipment works. Usually every bit of the equipment has not only been designed and built by the experimenters but is also maintained by them. Of course, there are some negative aspects. In a few places I noticed, for example, that resources appeared underused. But the main feeling is one of optimism.



Researchers at the Institute of Applied Chemistry, Kirin, discussing the structure and performance of a new polymer



The Academia Sinica at Guangzhou (Canton)

Most Chinese scientists (some 25,000 out of 36,000 of those in the Academia's institutes) were trained after the Liberation and before the Cultural Revolution. They were educated by teachers produced by the old social system; through remoulding and re-education, however, they are judged to have become "part of the proletariat" now that the gap between manual and professional labour is considered overcome. Many scientists are commended

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as advanced workers and are thus entitled to wage increments (the latest nationwide wage increase, on 1 November, was for 40% of state employees.) More important, more scientists are now allowed Party membership; they can be elected as deputies to the National People's Congress, or appointed to authoritative positions. In the past decade, a career structure, by qualification and seniority, has been designed and scientists can look foward to promotion.

A few institutions now adopt the convention that the authorship of papers will serve as a simple criterion of a member's contribution. All journals in China are, of course, published by the state and they have mandatory rankings, a paper in *Scientia Sinica* carrying the most weight. This may encourage the formation of self-centred sub-groups each pursuing prestigious research; the decline of collective authorship in the journals could be a reflection of this. More inventively, some institutions pursue the practice of arranging exhibitions of research results. All members are invited to assign 'work points' to the research on display according to personal judgements of its quality. Promotions are decided by the institution's 'academic committee' which refers to these collective evaluations. Some institutes also organise symposia at which their research is described and then discussed by co-workers invited from other institutes or universities.

Since 1978, scientists have been paid by the state for papers published whereas in the previous decade not even authors of books received royalties. Much stress is being laid on information science. Last August the China Society of Libraries was formed. Some of the larger university libraries plan to be computerised within a few years. Also in 1978 the Scientific and Technological Literature Press began issuing, for a whole range of subjects, monthly and bimonthly indices of foreign papers and specifications of open patents being applied for in Japan.

A national shortage of wood and wood pulp has constrained the publication of books and periodicals but is slowly being overcome. China now publishes a total of 1,200 periodicals in all areas and more will probably be launched. In electronics and communications technology, for example, there are some 20 primary journals with a general circulation. I also saw the following titles which are exclusively devoted to classical disciplines within physics: *Acta Physica Sinica, Wuli* (physics), *Acta Mechanica Sinica, Mechanics & Practice, Acta Acustica, Luminescence in Solids & its Application, Laser Journal, Gaofengzi Wuli* (high polymer physics), *Diwen Wuli* (low temperature physics), and *Physica Energiae Fortis & Physica Nuclearis*.

Students at the University of Science and Engineering, Tsinghua, engaged in an electronics experiment

Foreign journals are also used. Because of their high costs, however, most of them are centrally subscribed by airmail and then reprinted, the home-produced versions then being nationally distributed. The copies usually manage to reach the library shelves with no more than a few months' delay. There are no cover-to-cover translations into Chinese, and some readers may find this a problem.

The language problem can also affect visiting scholars. Speakers may find that their talks and the subsequent questions and answers are translated verbatim, sentence by sentence, with the help of the entire audience. Despite such difficulties, it is certainly a positive development that visits, in either direction, are increasing in both frequency and in the variety of disciplines covered.

'Centres of Scientific and Technical Exchange with Foreign Countries' have been set up in Beijing, Shenyang, Shanghai and Guangzhou (Canton) to assist the Foreign Affairs Bureau of the Academia Sinica and the Ministry of Education with scientific exchanges. The Scientific and Technical Association also oversees exchanges. Together with the US Committee on Scholarly Communication with the People's Republic of China (PRC), it is arranging for senior scientists as well as graduate students to stay for extended periods in China and participate in joint programmes. Sino-British cooperation in education, public health and culture was sealed by a five-year agreement signed at 10 Downing Street on 1 November 1979 during Hua's visit.

China now hosts international conferences more often, examples being the International Rice Research Workshop at the end of October last year in Guangzhou, and one on particle physics one month ago, at the same venue, under the chairmanship of Qian Sanqiang.

More than 1,600 academics, 180 research students and 420 undergraduates have been sent by China in the past two years to the US (500), UK (300), West Germany (200), France (200) and Japan (100). The overwhelming majority (1,800) are studying natural sciences. Advanced crash courses in languages, more often needed than not, are given just before departure or immediately after arrival at the host country.

Language will not, of course, be a problem for visitors of ethnic Chinese origin if they can speak the standardised dialect. Some of them have established close contact, and are on the 'academic committees' of some institutions. Scientists such as Chen-Ning Yang, Nobel prizewinner for the discovery of the violation of parity conservation, have made particularly valuable contributions, by participating in or advising on research, and inspiring young people, as well as cultivating friendship between China and their adopted countries.

'Sister universities' constitute another novel form of overseas ties. Zinghua at Beijing is affiliated to the University of California at Berkeley, Zhongshan in Guangzhou to the University of California at Los Angeles, and Nanjing to the University of Wisconsin. China is taking part in more bilateral and international scientific programmes. Recent examples are the measurement of $\nu_\mu e$ interaction cross-sections at the Fermi Laboratory and the Global Atmospheric Research Programme sponsored by the World Meteorological Organisation. As a result of these overseas links, increasing attendance at international meetings, and of submission's to western journals by Chinese scientists, it is now easy to find out who's doing what in the country. One source of information is the *Directory of Scientific Research Institutes in PRC*, produced by the National Council for US–China Trade in Washington, D.C. □

One man, two cultures, one discipline

Robert Walgate speaks to a former film-maker who subjected himself to the 'discipline' of science, and succeeded

"IT WAS a pretty nasty business" said Michael Shallis, gesticulating from his office chair against a blackboard backdrop of equations and graphs, "making cinema commercials. I spent a year as a staff film director for the Rank Organisation." He laughed. "It was really quite ludicrous — we'd make about six a day."

Dr Shallis is now a research fellow with Professor Donald Blackwell in the Department of Astrophysics at Oxford, making ultraprecise, painstaking measurements of the oscillator strengths of spectral lines of iron and other elements, to create a new basis for classical stellar spectroscopy.

He took no interest in science at school; avoided university; and joined a film school in London. He became an editor with Movietone News, the last of the cinema newsreel makers.

But during the sixties he bacame "exposed to science in one or two curious ways. I was absolutely spellbound by a series on television by J. Z. Young, the biologist — I've still got the little BBC two and sixpenny booklet that went with it. I found that the way he thought was very like the way I thought".

"In the film industry, the way I thought was very different from the people I was working with. I can't really explain it because I didn't at the time understand it myself."

Could he understand it retrospectively? Very diffidently Shallis said that he had an objective curiosity which he could see reflected in Young; whereas the people in the film business work mainly from their emotional rather than intellectual centres.

Shallis felt he wanted to make a film about the way science shaped us, how society might develop — but he found it difficult to interest a backer. "I spent quite a lot of time seeing if I could combine some sort of interest in the sciences with other things . . ."

Shallis's shift to the physically objective, the rational — wasn't that totally against the trend of the times, which was to abandon science and technology for the mystical?

"Yes, as that happened I did exactly the opposite. Then I reached a decision in 1970, when I was 27, that the only way I'd understand about science was by going and doing it."

The next thing was to work out how to do it, with A-levels in art and English. He started reading books on science, and began to see that he was more interested in cosmological and astronomical ideas than biological. Shallis was amazed to discover Professor Elton's conversion course from arts to sciences at the University of Surrey. He spent a year on that and emerged four years later with a first in physical sciences. Both courses have now closed.

There was a lot of spectroscopy in the course; he was interested in astronomy (he built a photometer for his telescope as a final year project); and when it came to choosing where to do research he chose Blackwell's group.

"I don't regret the decision. I think the work we do here is the most interesting in ground-based astronomy in this country. I'm a pragmatic person; so I'd always have been an experimenter or an observer. I was pragmatic in film-making too, interested in behaviour rather than inner psychological motives."

Wasn't the kind of science he was doing with Blackwell very painstaking and unspectacular? Wasn't the amount of emotional return that he could get from measuring the width of a spectral line pretty small?

"Well it was something I really needed to do. From the almost "anything goes" attitude within films, to the highly disciplined work that we do . . . I had to work very slowly and carefully, to learn that sort of rigorous discipline, which I'd never learnt in the arts at all. So I needed in the sciences very much to discover that rigour — in order, in fact, to release my creativity.

"The gap between the two cultures is probably widening. Up until the age of 27 I'd never met a scientist. At school they were completely a different breed. But now I've crossed the gap and discovered they are not a different breed at all, but actually doing the same thing. That has always interested me."

So why is the gap there? Partly for social and educational reasons — it's the way we are brought up; partly because scientists like it to be there. There's a sort of class distinction on both sides.

But also it's a matter of access; anyone can buy a book or a record. "You can go up to a man in the street and say have you seen the latest Woody Allen or have you heard about the Picasso exhibition at the Tate gallery and he'll say yes I have, I haven't seen it, but I know about it and I think Picasso's bloody awful; but if you ask him about the latest experiment in particle physics he'll look at you and gape and say, Ugh! At least the creative arts are accessible.

"People's hostility to science is growing and will continue to grow. As pollution and nuclear accidents and these things continue, hostility will grow and will grow rampantly.

"One of the problems scientists face is that they are so bound up in what they are doing, they generally fail to see why other people aren't. They find it very hard to see that other people don't necessarily find what they are doing fascinating. One sees this in the arts too. But it's more important in the sciences, because of the impact of technology on lives."

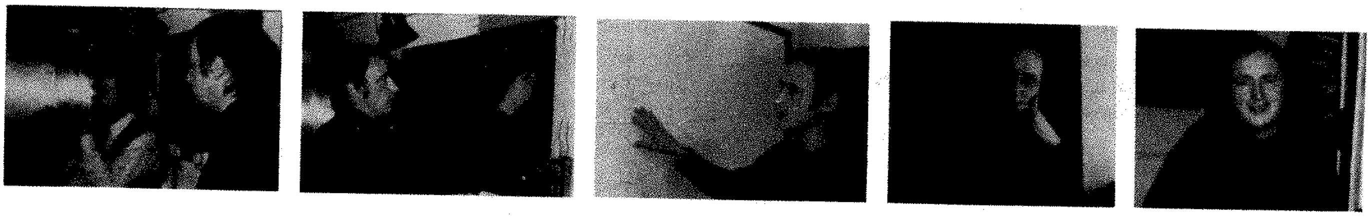
Now that Shallis has been through his creative period and his discipline he wants to loosen the discipline again.

"In a sense I want to integrate my experience. There are a lot of things I want to say about it. Which is why I want to move more into communication, writing books, teaching with the Department of External Studies. However fascinating research is, I still feel it is missing out on a lot of things, and there are other things I want to do.

"There are a lot of gaps to be filled in popularisation. The man in the street has very lopsided and wrong views about the nature of science, in ways that affect his life — like interpreting statistics.

"The thing that concerns me most within science itself is — I'm stepping carefully here — the lack of an ethical basis and the lack of a spiritual basis. It worries me very greatly.

"I came across a quotation from Jung last night: 'The rationalist unbeliever who has no gods left in his heart is tormented by the devils in his brain'. I think that's very good. Science as a collective thing has become a sort of rationalist unbelieving



Michael Shallis: film-maker turned astronomer

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entity with no gods left in its heart. And so it is tormented by the devils in its brain.

"One sees it in individual scientists to a greater or lesser extent. There's a scientist I know who believes — and it's a very widely held view — that ultimately everything in the universe can be reduced to physics. It is a very very sterile view, and one that I think is untenable. And in a sense their lives reflect this, their lives become extremely barren. Science is getting more and more materialist.

"Dora Russell said in her autobiography that science became inaccessible with relativity theory; and she saw that as the source of a lot of difficulties in the 20th century. Relativity theory was the first step towards obscurity."

How could ethics be grafted onto or grow out of science? "I'm sure there can be an ethical basis for science. The ethics have to come into the applications and the choice of science. It's tied up with the ethical basis of society."

Doesn't the scientist now have the ethics of the mercenary? Will he not do his science for whoever pays? "Yes. He thinks the freedom to investigate nature how he wants is his ultimate right. But I don't think anyone has ultimate rights.

"Take the use of animals. What ethical basis can you have for doing experiments with animals? The scientific community says animals should be available for whatever purposes we deem we need them for. We will be as humane as we can within those terms, but ultimately our interests over-ride the concern for the animals. I think that is a devastating ethic for science."

The discussions are always expedient, rather than ethical. "Of course that is a difficulty that our society in general faces — society doesn't have an ethic any longer that gives us any basis for our political judgements, our economic judgements, our personal judgements."

What about the spiritual element? "It is certainly lacking. Very few scientists have a feeling of awe about the universe; a few talk about it; but the whole thing becomes rather mundane for them."

But what should a group do? Every Saturday morning gather round and have a contemplation exercise? Shallis, laughing: "Maybe they should, quite possibly . . . . I haven't any answers, you see, I'm just beginning to become concerned with these things, and the more I think about it the more I become worried."

Does Shallis get any spiritual return from his spectral lines? "Out of science I get very little." But what kicks, I asked, *does* he get?

He is very enthused by the programme of Professor Balckwell's department. "Yes, that is very interesting, this is the kick, but so what? Can we justify that kick on other grounds? Is it enough? Matisse gets a kick out of doing a painting; a scientist out of his piece of science. On an individual basis that's fine. But what happens then? Science has, where art hasn't, an impact on the world. In science we have a whole group of people doing it for that kick, but we have also a vast power structure influencing the lives of all of us; and it is out of overall control. It is enough to Matisse; whether someone buys the painting or not is an irrelevancy to him; but the products of science are used and manipulated. I have no objection to the individual scientist; it is the influence of the collective. But then where does one draw the line?" ☐

Animal or vegetable?

THOMAS H. JUKES

VEGETARIANISM is a fascinating and delicate subject. It must be treated with tact because to many people it is of religious significance, but its scientific side compels interest and discussion. Bernard Shaw used to tell hostesses who invited him to dinner that he would not come to eat dead animals. He also refused to eat asparagus, which he said was a very nasty vegetable. His ebullient contemporary, GK Chesterton, in contrast, wrote some rollicking verses that made fun of non-meat-eaters, and non-milk-drinkers ("I will stick to wine and sherry, because they are so very, so very, very, very vegetarian").

An important step towards asserting the rights of plants was taken when it was found that cytochrome *c* of wheat and vertebrate animals had amino acid sequences so similar as to betoken the sharing of a common ancestor [1]. Indeed, the sequences of the cytochromes *c* show a phylogenetic relationship throughout the eukaryotes and extending to the purple bacteria. We cannot even eat a mushroom without devouring a relative. Some people talk and play music to their house plants, and we may soon hear of a Society For The Prevention Of Cruelty to Vegetables.

Deficiencies in foods of vegetable origin have been responsible in many countries for slow growth in children and, in consequence, for undersized adults. Even in the "developed" countries, it is sometimes ruefully pointed out that farm animals often receive better dietary treatment than human beings. This may be partly because cows do not read.

About 40 years ago it was evident that something in meat and fish, an "animal protein factor", was needed by chickens and pigs to correct a deficiency in diets consisting of foods of plant origin. Soybean meal, although a valuable food, lacked the missing substance. It was amusing and paradoxical that a vegetarian friend of mine, Lester Smith, successfully hunted down the missing substance in beef liver. In 1948, he isolated the anti-pernicious-anemia factor, which was also the animal protein factor. Simultaneously, the substance had been isolated from fermentation of a soil microorganism *Streptomyces griseus* [2]. The compound was was re-named "vitamin B_{12}", and the catchy designation did wonders for it. US professional football players, so I read recently, sometimes receive vitamin B_{12} injections to prepare them for the fray. Faith can move mountains.

Green plants do not make or need vitamin B_{12}. It all comes from microorganisms. Animals receive their supply from milk, or by predation, or from bacteria in the digestive tract, or by voluntary or involuntary coprophagy. The ruminating cow has an internal fermentation vat from which vitamin B_{12} gets in her milk to the benefit of lacto-vegetarians and other consumers of dairy products. Rabbits, less fastidious, consume their own faeces at night to obtain vitamin B_{12}. McGee [3] described the preservation, drying and consumption of faeces by the Seri Indians of Tiburon Island in the Gulf of California, who felt that endurance for the hard warpath on prolonged chase was augmented by this practice. Perhaps this was another case of faith moving mountains.

The consumption of meat is currently under criticism because its production is wasteful of food resources, such as cereal grains, that could be consumed directly by people. Against this, it is argued that grains are deficient in iron, and are poor sources of proteins. It is also true that ruminant animals supply us with meat and milk while consuming large quantities of forage and roughage, such as stalks and straw, that are not edible by human beings. The fermentation vat of the rumen converts the roughage to soluble nutrients. Some of the rumen organisms, *Methanobacteria* are strict anaerobes that may be "living fossils" of a very ancient era.

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(1) Stevens, F.C., Glazer, A.N., and Smith, E.L. *J. Biol. Chem.* **242:** 2764-2779, 1967.

(2) Rickes, E.L. *et al Science*, **108:** 634-636, 1948.

(3) McGee, W.J., *17th Annual Report, Bureau of American Ethnology*, Part 1, p.212, Washington, D.C., 1898.

CORRESPONDENCE

Future world energy needs

SIR, — It would have been most instructive if we had been told the composition of the "Conservation Commission" of the World Energy Conference, whose 1978 Report on Energy Requirements between 1985 and 2020 was reviewed in *Nature* (November 15, page 344). Neither the Commission nor your reviewer appear to have addressed themselves to the possibility of meeting their projected goals. To reach them the Commission says that nuclear generating capacity must be doubled every six years from 1985. If we suppose that there would be no more than 200 1000 MWe reactors operating by that date, this growth rate would mean that 12,800 reactors of the same size would have to be built by 2020. To reach this number the world would have to build a reactor a day from 1985 onwards. Furthermore, since reactors are unlikely to last more than about 35 years, by 2020, this rate of building would have to increase to two per day unless of course demand miraculously levelled off at this point in which case it would merely be necessary to go on building a reactor a day for evermore.

The 12,800 reactors to be completed by 2020 AD would, during their lifetimes, need to consume up to 140 million tonnes of uranium metal, which is 10-15 times the 1979 estimates for the world's stock of uranium costing less than $30 a pound to extract.

The UK government has recently given an estimate of $£8 \times 10^8$ for building a 1000 MWe nuclear plant. 12,800 reactors of this type would therefore cost a total in the region of $£10^{13}$ in 1979 values. This and the fact that at present in the US it takes about 10 years between design and commissioning of a reactor (and reactors become steadily more complex in an effort to meet ever-stricter safety requirements) appear to indicate that the world's attitude to energy consumption will have to change radically within a few years from now.

In assessing energy "demands" one must remember that a 1974 UN forecast was that average energy "demand" in the developed countries up to 2020 AD would rise thirteen times as fast as population, whereas it was expected to fall in the developing ones. Projections of energy demand are therefore merely estimates of how much energy people will be willing and able to buy: they have nothing to do with opinions about what distribution of energy consumption would be socially or morally desirable. Perhaps we should start asking ourselves such questions now?

Yours faithfully,

R. B. TEMPLE

Physical Chemistry Department, University of Sydney, Australia

Biotechnology report

SIR, — I should be grateful if I might make it clear to your readers that the Biotechnology Report partly published by you (24 January, page 324) was not "made available to *Nature*" by the Working Party, who were unaware that you had obtained an early draft until two days before its publication. It was an uncorrected, tentative draft, sent to our three sponsors (ACARD, ABRC, and the Royal Society), and to many of those who gave evidence to us, in November. Their advice led to significant alterations, only completed on 21 January What I hope will be the final draft has now been sent to the sponsors for approval of publication.

Yours faithfully,

ALFRED SPINKS

Wilmslow, Cheshire, UK

Short-term research contracts

SIR, — It is indeed heartening to read your valuable and informed coverage of the problems of experienced and accomplished scientists stranded and unemployed in their thirties and forties. It is equally disheartening that *Nature* is almost completely alone, both in the specialist and the national press, in drawing attention to the problem and its magnitude.

Why is everyone apparently so complacent? Are research directors and professors alike so committed to the exploitation of cheap and expendable labour that they see no need to speak out against a system whose iniquities they have been fortunate enough to escape and whose benefits they shamelessly enjoy? Why do we hear nothing from the heads of research councils and grant-giving agencies? It may be argued that the present situation has developed of its own accord. Does this mean that nobody should now begin to set matters right? Where does responsibility for perpetuation lie?

I issue a challenge, to the head of any research council or funding organisation, to defend the present system of short-term contracts in scientific research. I challenge him to defend the practice of signing away rights in common law as a prerequisite for the acceptance of such contracts. I challenge him to justify the expenditure of public money on the training and usage of men and women drawn from the most talented sections of the community, to be tossed into the dole queue before their full potential is realised.

Yours faithfully,

ROBERT JONES

Am Steinberg 67, Heidelberg, Germany

Pheasants and spies

SIR, — Having just returned from Kathmandu, Nepal, where a highly successful International Pheasant Symposium was held from 21-24 November, I was horrified to read your story headline 'India's wildlife projects under spy cloud' (29 November, page 435). In this story, under a cartoon depicting Kashmir pheasant beaters, you report that "Foreign collaborators might use the [World Pheasant Association's] pheasant project [for the re-introduction of the Cheer pheasant] as a camouflage for spying in the sensitive borderland".

Had you checked with the World Pheasant Association you would have discovered that the "foreign collaborators" for this project confine themselves to despatching batches of Cheer pheasant eggs from Heathrow to Delhi, where they are met by Indians, hatched by Indians and reared by Indians with the full knowledge and support of the state governments concerned; clearly they do not share your correspondent's fear.

To set up the project I have myself visited the Wildlife Departments and other officials in Lucknow (Uttar Pradesh), Simla (Himachal Pradesh) and Srinager (Kashmir), all leading tourist centres and none of them on the 'sensitive borderland'. The publication of unmitigated nonsense like this can do the work of recognised charitable conservation organisations like the World Pheasant Association and its objectives nothing but harm.

Finally, the World Pheasant Association received from the Chief Minister of Jammu and Kashmir an invitation to hold the Second International Pheasant Symposium in Kashmir, (an invitation that is being accepted). Hardly the action of a government concerned with spies infiltrating its territory.

Yours faithfully,

K.C.R. HOWMAN

World Pheasant Association, Suffolk, UK

Standardisation of research citations

SIR, — Three groups of life science editors have tried to achieve the standardisation of reference citation for which P.C. Reid argues so cogently (17 January, page 242).

First, in 1973 the biochemists (IUB-CEBJ)[1] published a reference list style suitable for journals adopting either the 'Harvard' system (names and dates) or numbers for citations in the text:

Abel, B.C., Cain, A.D., Adam, E. & Eve, A. (1976) *Tree J.*, **12**, 90-99

Second in 1977 ELSE (European Life Science Editors' Association) and the Ciba Foundation organised a workshop on references and produced a set of suggestions[2] for a style that is also suitable for either names/dates or numbers in the text:

Abel BC, Cain AD, Adam E, Eve A 1976 Bark thickness in apple trees. Tree Journal 12:90-99 (*or* Tree J 12:90-99)

Third in 1978 the International Steering Committee of Medical Editors proposed what is now known as the Vancouver style[3], suitable only for citation by numbers:

Abel BC, Cain AD, Adam E, Eve A. Bark thickness in apple trees. Tree J 1976;12:90-99.

The main differences between the first and second systems lie in the punctuation, the typefaces, and whether article titles are included or omitted. The main differences between the second and third systems lie in the way references are cited in the text and in the punctuation and position of the date in the reference list. All three systems recommend that the 'International List' (ISO 833-1974; BS 4148 Part 1; 1970 and Part 2:1975) should be used for abbreviating journal titles, in preference to the World List.

The ELSE-Ciba Foundation system suggests that journal titles might be either unabbreviated or abbreviated, provided that each reference list is self-consistent on this point. A later suggestion is that editors and publishers should allow both names/dates and numbers in the same journal or book, provided that each article or chapter follows one style consistently. Since the American National Standard (ANSI Z39.29-1977) and the draft International Standard (DIS 690) on bibliographic references both permit flexibility in the position of the various elements, including the date, in reference lists it would be a great step forward if the third system became more flexible on this, so allowing names/dates to be used in the text. The second and third systems would then be even more alike than they already are, and the work of authors, typists and typesetters would be enormously simplified. (Copies of the ELSE-Ciba Foundation system are available in either a full or an abbreviated form from the editorial department of the Ciba Foundation, 41 Portland Place, London W1N 4BN, to anyone enclosing a self-addressed adhesive label with their request.)

Yours faithfully,

MAEVE O'CONNOR

The Ciba Foundation, London, UK

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1. IUB-CEBJ. *Eur. J. Biochem.* 37, 201-202 (1973). (And elsewhere.)
2. O'Connor, M. *Editing Scientific Books and Journals*, p 176-185 (Pitman Medical, Tunbridge Wells, 1978). (And elsewhere.)
3. International Steering Committee of Medical Editors. *Br. med. J.* 1, 532-535 (1979). (And elsewhere.)

NEWS AND VIEWS

Communication between cells

from W.H. Evans

ANIMAL cells communicate in a variety of ways. The most direct and intimate communication must occur between assemblies of cells, allowing them to summate and coordinate their growth, development, division, metabolism, response to external stimuli and so forth. Exactly how this occurs is still a mystery, but as Unwin and Zampighi show on page 545 of this issue of *Nature* answers are beginning to be found at the cell's surface membrane — the repository of so many regulatory mechanisms.

A simple fact, appreciated widely only in the past 30 years — so persistent has been Schleiden's cell theory of 1838 emphasising that cells are self-contained independent units — is that most animal cells contain at areas of surface contact hydrophilic channels bridging the intercellular gap and directly connecting the cells' cytoplasmic milieu. These intercellular communicating channels can control the extent of equilibration of the cytoplasmic components, and they also provide the potential for the transfer or exchange of informational molecules across large sheets of cells — a property appreciated by developmental biologists. Provided that channel porosity is regulated, macromolecular individuality of the cooperating cells is maintained while the sharing of simple ions and molecules is permitted. The arrangement can appear as a sort of compromise between the independence seen in circulating blood cells and in nerve cells (which have developed alternative methods of communication using synapses) and the wholesale fusion of cells to form functional syncytia as seen in skeletal muscle.

By the 1960s a number of diverse biological observations converged to implicate plasma membrane junctions as the probable pathway for the rapid spread across tissues of injected fluorescent dyes, the passage of electrical current across heart tissue, and even the exchange of metabolic intermediates between cells in culture. Indeed, the last, termed metabolic cooperation (Pitts & Sims *Expl Cell Res* **104**, 153; 1977), has provided a most powerful experimental tool to investigate the specificity requirements leading to exchange of metabolites and has shown that channels are formed rapidly — within 30 min of intercellular contact (Epstein & Gilula *J. Cell Biol.* **75**, 169; 1977).

In 1966, Lowenstein (see *Biochim. biophys. Acta* **560**, 1; 1979) proposed a model for a direct cell–cell pathway that could permit ions and hydrophilic molecules of up to $\sim$1000 molecular weight to move more or less freely between cells. Soon after, the responsible anatomical structure in the plasma membrane of epithelial cells became clearer when Karnovsky and Revel used the lanthanum impregnation technique to distinguish clearly between tight and gap junctions between cells. The former seal off the intercellular space, whereas the latter have a 20–40 Å gap permeable to lanthanum. The use of the term 'gap junction' is perhaps unfortunate, for the intercellular gap revealed by electron-lucent tracers is bridged by leak-proof channels connecting the cell interiors. The functional distinction between these often-confused cell junctions could not be greater. Fast on the heels of the identification of gap junctions in tissue thin sections was the demonstration, by freeze fracturing the component plasma membranes, that gap junctions are easily identified as plaques of closely aggregated intramembranous particles. This quickly led to gap junctions being recognised throughout phylogeny — from simple coelenterates, such as *Hydra* to man — providing compelling evolutionary evidence for their functional importance.

The lattice-like nature of gap junctions is conspicuous when the junctions, purified from tissues, are viewed in the electron microscope after negative staining. Their striking hexagonal packing makes them attractive candidates for ultrastructural analysis by non-destructive electron microscopy and image reconstruction — approaches which have been developed by the Cambridge group using bacterial purple membranes. High resolution electron microscopy and X-ray diffraction have already provided plausible models of hepatic gap junctions in which proteins of the apposed plasma membranes are arranged hexagonally producing a channel traversing the membrane (see Bennett & Goodenough, *Neurosci. Res. Progr. Bull.* **16**, 375; 1978). In this issue Unwin and Zampighi put forward a new refined model to explain junctional communication between cells across hepatocyte gap junctions, the largest biomembrane channels known.

By tilting negatively-stained junctions in the electron microscope, and analysing the pictures using Fourier and image reconstruction techniques, the communicating unit (connexon) emerges as an hexameric polymer of six subunits spanning the membrane and protruding from either side. The subunits are tangentially inclined which explains the annular appearance of each connexon. The channel is approximately 20 Å wide at its exposed region, although the channel dimensions deeper inside the 'tunnel' are not resolved. Models of gap junctions should explain how channel porosity can be regulated between open (cells are coupled) and closed (cells are uncoupled) configurations, an important physiological function sensitive to changes in Ca^{2+} concentrations and/or *p*H. Unwin and Zampighi's model provides a neat and simple explanation for changes in channel porosity on the basis of maps of two junctional states by postulating a rotational displacement of the hexameric subunits, so opening and closing the narrow channel.

Models of junctions between animal cells have leapt ahead of our knowledge of junctional biochemistry and the nature of ions and molecules transferred in specific functional states. Crystalline lattice structures are often the outcome of molecular parsimony, and although it is often assumed that the hepatic gap junction, like the purple membrane, is composed of a single monomeric polypeptide, the biochemical evidence is still unsatisfactory. The lipid composition is unexceptional (Hertzberg & Gilula *J. biol. Chem.*, **254**, 2138; 1979), and the isolated junctions, prepared by detergent extraction of plasma membranes, are strictly lipid depleted, detergent-binding fragments.

Some clues have recently emerged implicating cyclic nucleotides as possible important metabolic control signals transferred across junctions. It was shown by Lawrence *et al.* (*Nature* **272**, 501; 1978) that hormonal stimulation can be transmitted between cocultured granulosa and myocardial cells. The cells were joined by gap junctions and since hormonal stimulation of both cell types is cyclic AMP dependent, it is possible that cyclic AMP is the agent responsible for cross-stimulation. Cyclic nucleotides may also feature in intercellular communication in heart tissue where synchronously contracting cardiocytes are joined by intercalated disks which incorporate gap junctions. The possibility arises that cyclic nucleotides first arose primordially as

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W.H. Evans is at The National Institute for Medical Research, Mill Hill, London.

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signal molecules sufficiently tiny to move directly and rapidly across junctions between cells before they also became implicated in hormone receptor-mediated communication between organs. In the same vein are reports of a correlation between gap junction formation and hormonal activation in myometrium (Garfield *et al. Science* **198,** 958; 1977) and oocytes (Browne *et al. Science* **203**, 182; 1979). And finally, to the transmission of hormonal stimuli between cells connected by gap junctions one can also add that the action of interferon is also likely to be mediated and amplified by the same intercellular pathway (Blalock & Stanton *Nature* **283**, 406; 1980). Clearly, now that gap junctions have emerged as widely-distributed plasma membrane organelles tailored for intercellular communication, the finer detail of their biological role still remains a wide-open question. □

Information exchange in the Palaeolithic

from Clive Gamble

DEVELOPMENT in palaeolithic culture can no longer be adequately explained in terms of increasing intelligence, for recent studies have demonstrated the existence, some 300,000 years ago, of mental ability equivalent to that of modern man. The dramatic developments in material culture, such as the appearance of art, now seem to be more closely related to changes in the amount and kind of information needed by palaeolithic societies, rather than dependent on the evolution of the brain.

Archaeologists have generally concentrated on the obvious in their studies of the earliest art. Understandably they have felt most at home when discussing the representational images that were either painted, sculpted or engraved by palaeolithic man. The renderings of animals and humans have been analysed in terms of composition and the chronological development of artistic styles. Their visual content has been interpreted in terms of either magical or religious significance. These images are seen as a key with which the archaeologist can unlock the psychology of early man while also providing an opportunity to investigate the cognitive development of the genus *Homo*.

These painted bison and carved figurines are however only part of the artistic output of palaeolithic man. A large body of less obvious art has also been recovered and consists of scratches on ornaments, pieces of bone, stones, the walls of caves and impressions drawn in clay. Their interpretation has barely proceeded beyond regarding them as the first 'doodles'; an analysis that stems from their apparently random execution. In a series of recent papers Alexander Marshack has subjected these scratches to a detailed systematic analysis (*Curr. Anth.* **20,** 271; 1979; *Form in Indigenous Art* (ed. Ucko) Australian Institute of Aboriginal Studies 1977). His method of investigation involves microscopic examination of how these abstract images were made. His studies have revealed that far from being random these scratches were formed in an intentional sequence whereby composite abstract images were built up. He has further grouped the scratches on the basis of repeated motifs into descriptive classes such as meanders, fishilike images, zig-zag and parallel lines.

The majority of these scratched signs and their painted counterparts on cave walls are found associated with upper palaeolithic stone tool assemblages. They appear some 32,000 years ago at a time when the fossil record indicates the replacement of *Homo sapiens neanderthalensis* by *Homo sapiens sapiens*. This is also the period when the first representational art is found in Europe. However Marshack has also been able to show that in two instances the same intentionality in scratching signs predates the appearance of modern man. This important finding suggests that the potential for image composition of this kind was possessed by both neanderthal and *Homo erectus* populations. This raises the possibility that the traditional explanation for such behaviour, which regards art as a unique developed mental ability of *Homo sapiens sapiens*, is no longer entirely adequate. Wynn (*Man* **14,** 371; 1979) has analysed the operational intelligence of handaxe manufacturers from Isimila, a site comparable in age to Pech de l'Azé where Marshack has documented the first intentional scratched meander. His analysis uses Piagetian genetic epistemology as a framework to investigate the cognitive procedures involved in the manufacture of these artefacts. On the basis of this study Wynn concludes that the hominids who made the handaxes were not significantly less intelligent than any modern adult. His important conclusion is therefore that increasing intelligence cannot be used as an explanation for developments in material culture over the past 300,000 years.

These provocative studies challenge what has to be explained by palaeolithic archaeologists. If we accept their conclusions then it becomes clear that it is no longer sufficient to look at human evolution over the past 300,000 years as simply a process of increasing intelligence and offer explanation in terms of changes in the brain. Instead we must approach the problem from a different perspective and look for changes in social circumstances which selected for and brought into play these latent patterns of behaviour.

One current line of enquiry stresses the role of information exchanges in palaeolithic society. In this context art and the scratched signs become part of a visual system of communication designed to handle information in this mode. The sudden appearance of art in the upper palaeolithic is thus a response to handling more and different types of information. Style provides the means by which order is brought to this form of communication so that messages can be sent and received. All items of material culture can be modified by stylistic rules to provide such additional visual information. This perhaps explains why there is such a sudden increase in stylistic behaviour among the stone tool assemblages at the same time as the art first appears since, as Wobst (*Papers for the Director* (ed. Cleland) Anthropology Papers, **61,** Museum of Anthropology. University of Michigan, 1977) has pointed out, if the communication system is to function without ambiguity then either everything or nothing must be used to transmit information in this fashion. Moreover, the organisation of the system according to symbols would also lead to a greater visual economy and an ability to make the system compact and easily manageable. A limited range of symbol/signs can be used to code a great variety of messages and would prevent the system from becoming too unwieldy if only representational images were used. Conkey (*Social Archaeology* (eds Redman *et al.*) Academic Press, 1978) has proposed that it is in the upper palaeolithic that the first pictorial symbol systems are found and which indicate that information was being organised according to this mode. The scratched motifs identified by Marshack from this period are most probably examples of such symbol systems.

But why should these hunter-gatherer societies have needed new means to exchange information about membership, status and position? Is it in fact possible to demonstrate changed social circumstances that would have selected for visual systems of communication? If we concentrate on the appearance of art and symbol systems and assume that these were a response to new social conditions then the argument becomes very circular. Where it might be possible to see changes is in the external circumstances of these societies. The first of these could relate to climatic changes at the time the art first appeared. It is first found when the climate of the last ice age turned towards much harsher, colder conditions that culminated in the

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C.S. Gamble is in the Department of Archaeology, University of Southampton.

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maximum extensions of the icesheets some 18,000 years ago. This would have resulted in groups becoming more dispersed as they adjusted to the new environments and the resources they contained. There would have been considerable advantages in seeking further ways of signalling membership in order to link such groups together. However this must have been a problem that faced palaeolithic groups during the earlier phases of the European ice ages and yet they did not produce art as a response. The second factor in the equation most probably concerns population. Hassan (*Advances in Archaeological Method and Theory* (ed. Schiffer) **1**, Academic Press, 1978) has estimated both higher annual growth rates and greater population densities between the upper and middle palaeolithic. If this was the case then there must have been considerable pressure to hive-off population into new territories as well as to maintain growth rates at accustomed levels in just those areas that were now being affected by climatic change. The extra information would therefore be needed to relate groups to resources and to define the extra population. Verbal communication may previously have been sufficient to exchange information but now the possibility of language differentiation would arise and this could most economically be averted by instituting a new form of communication based on visual messages that were coded stylistically. It is noticeable that at this time global population increased its range with groups colonising Australia, central Asia and hence ultimately the Americas.

The proposition that art and the intentional scratches are part of a system of communication does raise many interesting possibilities in terms of what changed in the course of human development. It also suggests that archaeologists recast their explanations for variability in the palaeolithic record toward questions concerning information rather than intelligence. □

Possible hazards of photochemotherapy for psoriasis

from Bryn Bridges and Gary Strauss

THE photosensitising psoralen drugs are being increasingly used in combination with near-ultraviolet light (PUVA) as a very effective treatment for psoriasis and other skin conditions. PUVA controls excess cell division in the skin by virtue of its ability to damage DNA. In experimental systems PUVA lesions are able to elicit all the usual consequences of DNA damage, for example inhibition of DNA synthesis, mutation, chromosome aberrations and cell transformation, as well as initiating skin tumours in mice (see Bridges *et al. Clin. exp. Dermatolol.* **3**, 349; 1978; Vella Briffa & Warin *J. Roy. Soc. Med.* **72**, 440; 1979). The possibility that it might initiate skin cancer in humans was much in the minds of dermatologists; indeed the UK Medical Research Council has set up an *ad hoc* Working Group to consider the possible hazards of PUVA.

Although natural plant sources of psoralens have been used to treat vitiligo (lack of skin pigmentation) for thousands of years, the first report of their carcinogenicity in man followed PUVA treatment of some patients suffering from xeroderma pigmentosum, a condition in which the cells cannot repair damaged DNA effectively. Skin tumours began appearing within a month of initiation of treatment and one patient developed a lymphatic leukaemia (Reed *Acta Dermatovener. (Stockholm)* **56**, 315; 1976; Reed *et al. Arch. Dermatolol.* **113**, 1561; 1977). The surprisingly rapid appearance of the tumours was disturbing and unexpected for they appeared too soon after treatment to be readily explained by an increased rate of tumour initiation *de novo*. More recently an increased rate of skin cancer has been reported within the first two years of PUVA treatment of psoriasis patients (Stern *et al. N. Engl. J. Med.* **300**, 809; 1979). Although the magnitude of the increase is arguable (no matched control group was used, it being a longitudinal 'before and after' comparison) it is clear that the excess tumours are associated with the previous history of the patient, either of radiation exposure or previous skin cancer.

Taking these two sets of observations together, it seems that PUVA is acting not only as a direct initiator of skin tumours (though this has yet to be established in man) but as a type of so-called 'promoter', allowing the full expression of potentially malignant foci. The idea of promoters arose from observations that some chemicals, notably the phorbol esters, although not mutagenic or carcinogenic in themselves increase the incidence of tumours if given after a very low dose of a known carcinogen (see for a short review Marx *Science*, **201**, 515; 1978).

Support for the idea that PUVA, although potentially mutagenic and carcinogenic in itself, is acting in this case as a promoter comes from experiments in which ultraviolet-induced skin tumours were transplanted to syngeneic mice. The tumours grew in mice treated with PUVA but not in untreated mice (Spellman *N. Engl. J. Med.* **301**, 554; 1979).

How could such a promoter effect be operating in this case? One possible mechanism is the suppression of immunological surveillance in the skin. The idea that the body has a natural surveillance mechanism by which cells of the immune system recognise and destroy cancer cells is now generally discredited. But the skin may well be an exception to this rule. The demise of the immunosurveillance theory followed observations that there was no general increased incidence of tumours in immunosuppressed patients; however, although suppression of cell-mediated immunity by drugs such as azothioprine does not lead to a general increase in cancer, there is a rise in the number of lymphoreticular tumours and of squamous cell carcinomas of the skin (Walder *et al. Lancet*, Dec. 11, 1282; 1971; Kinlen *et al.*, *Brit. med. J.* **2**, 1461; 1979). In Stern *et al.*'s study cited above, the excess tumours were also squamous cell carcinomas whereas basal cell carcinomas predominate in the general population. This raises the possibility that PUVA may be immunosuppressive in man. In guinea pigs PUVA treatment of skin blocks delayed hypersensitivity responses in exposed areas and to a lesser but quite significant extent at unexposed sites (Morison *et al. J. invest. Dermatol.* **72**, 273; 1979). The cause of the immunosuppression is as yet unknown. Roberts and Daynes (quoted by Spellman, above) have recently reported that treatment of mice with PUVA induces the development of suppressor cells. In man, a significant decrease in T cell number and function has been reported in patients undergoing PUVA therapy (Cormane *et al. J. invest. Dermatol.* **68**, 253; 1977; Morison *et al. J. invest. Dermatol.* **70**, 216; 1978). There is no doubt that in man circulating lymphocytes are exposed to substantial doses of PUVA when passing through the skin of treated psoriatics (see Strauss *et al. J. invest. Dermatol.* **73**, 211; 1979). Furthermore, there are telling arguments in favour of the existence of resident lymphocytes in the skin (see for example, Streilein *J. invest. Dermatol.* **71**, 167; 1978).

That the promoting effect of PUVA is indeed a consequence of DNA damage is indicated by rather similar immunosuppressive effects being reported for far ultraviolet light (Daynes *et al. Transplantation* **23**, 343; 1978; Kripke & Fisher *Natn. Cancer Inst. Monogr.* **50**, 179; 1978) and by the very much more rapid appearance of tumours observed in PUVA-treated xeroderma pigmentosum patients compared with individuals with normal DNA repair ability. The target cells for the PUVA-induced immunosuppression seen in rodents are presumably different in type from those in human psoriatic plaques that respond to the therapeutic effects of PUVA, but whether the immunosuppressive response is specific for DNA-damaging treatments or whether

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Bryn Bridges is Director of the MRC Cell Mutation Unit and a Professor at the University of Sussex; Gary Strauss is supported by the Cancer Research Campaign at the University of Sussex.

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it could be elicited by other agents using other mechanisms of cytotoxicity, is an open question.

Taken together these observations suggest several possibilities of considerable fundamental as well as clinical importance. (1) Immunological surveillance may well be a reality in the skin; (2) immunological surveillance in the skin may be suppressed by DNA-damaging agents; (3) if PUVA is both a promoter and an initiator, its long-term sequelae in man may be even more severe than has hitherto been suspected.

Finally, the results with PUVA must inevitably lead to an examination of the effects of naturally occurring psoralens present in some sun-tan preparations since the activity of these in sunshine must surely result in DNA damage to the skin (Ashwood-Smith *Brit. med. J.* 3 Nov. 1140; 1979). ☐

The case for layered convection

from Peter J. Smith

INTEREST in the possibility of mantle-wide convection has revived recently, sparked off largely by the conclusion, derived most impressively by Cathles (*The Viscosity of the Earth's Mantle,* Princeton University Press, 1975), that contrary to previous widespread belief the viscosity of the mantle does not necessarily increase significantly with depth. With one of the most important restraints to the continuation of convection into the lower mantle removed, support for convection limited to, say, the asthenosphere is weakened, and a return to the earliest concepts of mantle-wide flow becomes possible.

Or does it? In a most thoughtful paper Richter (*J. geophys. Res.*, **84,** 6783; 1979) points out that viscosity is not the only criterion to be applied to the case for and against crust-to-core convection cells. Whatever the hypotheses about variation of viscosity in the mantle, it is an inescapable fact that earthquakes never occur below a depth of about 700 km, implying strongly that in trench zones (where the deepest earthquakes occur) the subducting lithosphere cannot pass into the region below 700 km. When an increase in viscosity with depth was accepted, this resistance to penetration seemed hardly surprising; but with that limitation removed, some other explanation of the barrier is required.

And the phenomenon is even more remarkable than a simple 700 km limit would suggest. From a new analysis of earthquakes in the Tonga–Kermadec subduction zone, Richter not only shows that all foci are shallower than 700 km but demonstrates (using focal mechanisms) that where shocks occur below 600 km the corresponding sections of the downgoing slab become subject to downdip compression. This confirms for a particular case a generalisation made earlier by Isacks and Molnar (*Rev. Geophys. Space Phys.*, **9,** 103; 1971) and strengthens support for the view that subducting lithosphere meets insurmountable resistances when approaching 700 km depths. So, indirectly, does Richter's discovery that the seismic energy released in the Tonga–Kermadec region decreases generally with depth but then increases phenomenally between 500 and 700 km — that is, in the zone immediately above the seismic cutoff. The significance of this increase is that it more or less rules out non-barrier explanations of the 700 km limit. It is possible to argue, for example, that subducting lithosphere does pass into the region below 700 km but that by then it has become sufficiently warm to deform by creep rather than brittle fracture, thereby eliminating earthquakes. But it is difficult to imagine a deep energy peak arising from a lithosphere gradually becoming hotter with depth. Similarly, it has been suggested that lithosphere passing through the 650+ km zone changes phase, giving rise to a ductile high-density form incapable of supporting earthquake mechanisms. This proposal is more difficult to evaluate; but again the energy release peak at depth poses a severe problem.

So the 700 km limit must represent a real barrier to the passage of lithosphere, for the large energy release in the 500–700 km interval can only be explained in terms of large compressive stresses resulting from the approach towards an irresistible obstacle. What is that obstacle if it is not a viscosity increase? It could easily be chemical inhomogeneity, claims Richter, who envisages a chemically layered mantle with density increasing with depth from layer to layer. If this sounds implausible, the converse — a homogeneous mantle — sounds even more implausible, at least as Richter explains it. If the mantle is homogeneous, he argues, it must have been homogeneous right from the start or it must have become so by thermal convective mixing. But mantle conditions are such that thermal mixing will only remove inhomogeneities of less than about 1%. In other words, the conditions for the occurrence of a homogeneous mantle are more restrictive than those likely to give rise to a mantle with more than minimal inhomogeneities.

Convective systems with chemical density variations have been studied in considerable detail in seawater. Extending the principles thereby derived to the mantle, Richter concludes that a significantly inhomogeneous mantle will develop layering in which convection is restricted to the discrete layers. Precisely how many convective layers there are in the mantle will depend on how the Earth accreted and how the mantle developed originally as the residue left by the removal of crustal and core materials — and these factors are uncertain. But since the mantle is more likely to be inhomogeneous than homogeneous, it is more likely to have more than one convective layer than it is to have just one. So, although whole-mantle convection is a plausible option again thanks to revised views about the mantle's viscosity, the details of the convection could be rather different from those envisaged by the early supporters of the idea. ☐

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

Antigenic shift and drift

from a Correspondent

INFLUENZA has been relatively dormant as a human disease for the past several years. However with the advent of recombinant DNA technology and of monoclonal antibodies, work on the influenza virus has burgeoned. Particularly exciting are the recent advances in knowledge of the structure of the viral haemagglutin (HA) and of the mechanisms which allow its antigenic variation and the consequent emergence of new viral strains. So it was not surprising to find that this molecule was of central interest at a workshop* held early in December near Canberra, Australia.

HA, the major glycoprotein of the viral envelope (molecular weight 75,000) is coded by an mRNA specified by one of the eight segments of RNA present in the virus particle. Pandemics of influenza occur in the human population when a major shift in the antigenic structure of HA allows the virus to infect people who are immune to previously circulating strains. Obviously one would like to compare the complete primary amino acid sequence of HAs of several different pandemic strains. However, despite considerable effort by the protein chemists (M. Waterfield, ICRF; C. Ward and T. Dopheide, CSIRO, Melbourne) such information has proved difficult and laborious to obtain. Consequently the nature of the variation involved in the creation of new pandemic strains has remained unknown. As reported at the workshop, several groups now have cloned in prokaryotic vectors, full length cDNA copies of the HA genes from viruses belonging to two of the three major pandemic groups. Complete DNA sequences have been established for the Asian H_2 strain, which first emerged in 1957 (M-J. Gething, ICRF); for several members of the Hong Kong series which first appeared in 1967 (N. Sleigh & G. Both, CSIRO, Sydney; W. Min Jou, Ghent; G. Threlfall, Searle) and for the Rostock strain of avian influenza virus (A. Porter & J.S. Emtage, Searle, High Wycombe). In all cases the general organisation of the HA gene is remarkably similar. There are untranslated regions (albeit of varying lengths) at both 5′ and 3′ ends of the gene. The 13 nucleotides which lie at the 3′ ends are identical in all viruses so far examined and are presumably used in replication (J. Skehel, NIMR, London); the remainder of the untranslated regions show little evidence of common origin or function (G. Air, Australian National University, Canberra). In all cases the coding portion of the gene exists as a continuous block of nucleotides so that removal of intervening sequences apparently is not involved in the expression of HA. However the mRNAs of influenza are known to sequester 5′-capped termini from nascent or newly-synthesised cellular RNAs, and splicing, or something like it, may play a part in this transfer (R. Krug, Sloan-Kettering Institute, New York).

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*An International Workshop on 'Structure and Variation in Influenza Virus', organised by Graeme Laver and Gillian Air, Australian National University, Canberra, Australia.

The sequence of amino acids, deduced from that of the DNA, confirms that HA invariably contains three distinct hydrophobic regions. One of these forms the N-terminus of the primary translation product. It is cleaved from the mature protein and is believed to be responsible for insertion of the nascent polypeptide into the membrane of the endoplasmic reticulum. A second hydrophobic region located at the C-terminus is involved in anchoring the mature HA in the viral envelope. Both of these hydrophobic regions show a surprising degree of variation and it seems that hydrophobicity *per se* is more important than primary amino acid sequence for interaction of HA with membranes. By contrast, the amino acid sequence of the third hydrophobic region which lies roughly in the centre of the molecule is absolutely conserved between viral strains. Ultimately it forms the new N terminus of the smaller subunit of HA (HA2) which is created by proteolytic cleavage of the full length polypeptide. This cleavage, which is necessary for infectivity of the virus, is thought to allow the third hydrophobic region to interact with the membrane of the target cell. Approximately 60% of the amino acid sequences of HA2 are conserved between strains. The remainder of the molecule (HA1) shows much more extensive variation. Nevertheless, because the locations of bridging cysteine residues in both HA1 and HA2 are totally conserved, it is likely that the three-dimensional structure of HA (currently under investigation, D. Wiley, Harvard University) will turn out to be very similar in different viral strains. These results are consistent with the idea (D. Jackson, University of Melbourne) that the major antigenic sites are located in HA1 and that it is variation in these sites which allows the virus to escape from neutralising antibody. However the results reported at the workshop show the HA1s of different pandemic strains to have diverged to such an extent that it is impossible to map their sites of antigenic difference merely by comparing their amino acid sequences. Furthermore the fact that the HAs of two human strains are no more similar to each other than to the HA of the avian strain means that it is impossible to establish a genealogical tree which would define the evolutionary relationship between different pandemic strains.

At present therefore, it is impossible to decide between the two alternative hypotheses which attempt to explain how new pandemic strains of influenza virus arise. One suggestion, for which circumstantial evidence exists (see Laver & Webster *Brit. Med. Bull.* **35**, 29; 1979) is that new pandemic strains are recombinants formed between influenza viruses of humans and those of domestic or wild animals. The second possibility is that the emergence of 'new' pandemic strains in the human population represents no more than the reappearance in essentially unchanged form, of viruses that had circulated years previously, and then had entered a latent state in humans or lower animals. Although such a latent state is entirely conjectural, this idea has received serious consideration because of the reappearance in 1977 in China of a strain of influenza virus that was in wide circulation until 1950 and then had disappeared. The re-emergence of this strain adds an entirely unexpected complication to both the epidemiology of influenza and the evolution of the virus. It implies that influenza virus, at least in some circumstances, in some hosts, can preserve its antigenic structure for long periods of time. Such is not the general case in humans.

Between the major shifts in antigenic structure which define the beginning and end of each pandemic era, the virus undergoes a series of smaller changes. As the human population becomes immune to infection by extant strains of influenza virus, so the pressure rises to select variants which by displaying small but significant changes in antigenicity can evade the immune response. This process is known as antigenic drift.

The changes which are responsible for antigenic drift accumulate with time, and field strains isolated several years apart from within a single pandemic era show a surprising number of nucleotide and amino acid substitutions (Both), in one case 20 amino acids in 9 years (Air & Laver). Presumably some of these substitutions affect viral antigenicity. However it is also possible that some of these changes may be antigenically neutral or may cause antigenically significant conformational alterations in distant parts of the HA molecule. Therefore, it has not been possible so far to correlate the locations of antigenic sites with the positions of amino acid substitutions.

To simplify the system, several groups have attempted to mimic antigenic drift *in vitro*. The virus is grown in the presence of large quantities of either high titre neutralising antibody (B. Moss, CSIRO, Sydney) or more recently, monoclonal antibodies directed against single antigenic sites in HA (R. Webster, St. Judes Children's Hospital, Memphis; Laver; W. Gerhard, Wistar Institute, Philadelphia). Variants which are resistant to inactivation by the antibodies arise at a frequency of 10^{-5} to 10^{-6}. Peptide mapping and nucleotide sequencing show that such variants arise as a consequence of changes limited in both number and location within HA1. Thus in 10 variants selected with monoclonal antibodies, a proline at position 143 of HA1 has changed to serine, threonine, leucine or histidine. The simplest interpretation of this result is that the region around position 143 is at or near an antigenic site. This is consistent with the finding that field strains which presumably have been undergoing a similar selection process *in vivo* often display changes in the same region of the protein, although for reasons which are not clear the proline at position 143 is invariant.

As stated above, the field strains show alterations in many additional regions of the HA molecule. Thus the total number of antigenic sites may be greater, their topological relationship more complex and the selection pressures which mould them more subtle than we at present appreciate. Nevertheless the capacity to select a sequential series of isogenic mutants and analyse them rapidly at the molecular level is an important advance that represents the best hope of dissecting the process of antigenic drift. ☐

Man in space

from Anthony Goode

MANNED space flight now spans almost two decades but it began amidst considerable scepticism; an Astronomer Royal summed up a widely held belief by stating, somewhat incautiously, that space travel was utter bilge, and certainly in 1961 extensive and catastrophic effects of exposure to weightlessness were predicted. Despite this, man is now firmly committed to space. Exploration of its full potential depends on his well-being in this unique environment. The reusable Space Shuttle, whose first flights are planned for 1980, will open up space to a wider range of users, including the working scientist and provide a chance to assess more clearly the potential medical problems.

Cardiovascular changes

Although the catastrophic effects of exposure to weightlessness have not materialised gross changes do occur in the

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bodies of astronauts. Going up into orbit immediately induces a massive redistribution of about 2 litres of blood and interstitial fluid into the head and thorax causing venous distension. The face becomes puffy whereas the legs shrink. This redistribution directly influences cardiac activity and indirectly, the control of the circulation, and produces changes in the clearance of salt and water through the kidneys. This thoracic engorgement persists in flights of up to 84 days giving rise to fears about the longer term possible consequences of cardiac hypertrophy and arterial hypertension. The changes however, do not affect work capacity in orbit. Return to Earth results in a decline in circulatory tolerance to an erect posture, reduced cardiac output and stroke volume and a decreased capacity for physical work. After Skylab flights these changes were reversed to normal pre-flight values within 10 days. The precise degree of thoracic engorgement and how close the pressure levels in the pulmonary artery come to inducing pulmonary oedema will be investigated early in the Space Shuttle series.

Changes in red cell morphology have been found after spaceflight together with loss of up to 8–10% of red cell mass after Apollo and Skylab missions. The reason for this is obscure; chromium-51 labelling of red cells has shown no increased destruction. Cell proliferation in tissue culture is normal in flight and the cellular and hormonal components of the immune response *in vivo* remain unchanged. Renal function is satisfactorily maintained but a possible diuresis early in flight is accompanied by a fall in antidiuretic hormone and an increase in aldosterone with paradoxical solute excretion.

Bone and muscle

In the first days in orbit there is a 3-4 kg loss in body weight as a result of loss of body fluid and, to a lesser extent, a reduced fluid intake. After a longer period in flight, 2–3 kg are lost, mostly as body muscle, although this loss may be reduced by about 50% by an intensive excercise regimen. The loss does not therefore simply reflect muscle atrophy, but no additional cause has yet been found. Bone calcium is lost continuously at a rate of 0.5% of total body calcium per month, about three times the loss induced by prolonged bed rest. Apart from increasing the risk of renal calculi in astronauts, this excretion could limit flights to about a year's duration before bones become to brittle to support the body on Earth. Rigorous excercise regimes, hormone analysis and dietary measures have failed to elucidate the underlying cause, and muscle and bone metabolism will be an area of intensive future investigation.

Man has a well-developed skeleton and musculature suited to Earth's gravity but such attributes may be an embarrassment in space. One observer has speculated that young amputees may be ideal crew members for longer flights, as they would be subject to less fluid shift and mineral depletion.

Sensory systems

On Earth man maintains his sense of balance by the gravity-sensitive vestibular system in the ear, which together with the somatosensory and visual systems allow spatial orientation and the control of posture and locomotion. However, in zero *G* the gravity-sensitive otoliths of the vestibular system fail to indicate the orientation of the head as indicated by the other sensory mechanisms and this mismatch causes a perceptual conflict during movement, either of the whole body or of the head alone. In zero *G* astronauts have experienced perceptual deficiencies or illusions such as a compelling sensation of flying upside-down or of turning or tilting when they move their head rapidly. Skylab astronauts reported that they were less able than normal to locate objects relative to their body when their eyes were closed.

Early flights in Mercury and Gemini capsules in which astronauts were restrained on couches and unable to move their body and head freely prevented the space motion sickness which had been reported during the Russian Vostok flights. However, during Apollo and Skylab missions half the crewmen developed sweating, pallor, nausea and vomiting, which began within minutes to hours of their starting to move around in the capsule and which remitted within 3 to 6 days. The phenomenon is probably due to intersensory mismatch and once the body has become accustomed to the new environment, head movements no longer cause nausea. Anti-motion sickness therapy may relieve the symptoms but the undesirable sedative side effects can result in less efficient performance during critical early phases of the mission. NASA has selected an experiment for Spacelab 1 in which astronauts will be exposed to a selection of motion sickness tests first on Earth and then in zero *G*. These tests should provide useful predictions of individual susceptibility to space motion sickness. The European Space Agency (ESA) has designed a linear accelerating stimulus device — the 'space sled' — which will allow studies of human sensory physiology, measurement of perceptual thresholds and analysis of eye movements and muscle activities affected by defined rectilinear accelerations.

Radiation hazards

Certain components of cosmic radiation may be a hazard to man in space. The Apollo 11 crew saw faint spots and flashes of light every half minute or so on the return flight from the Moon. This was the result of HZE particles (of high charge and energy up to 10^{14} MeV) penetrating the spacecraft and the astronauts' eyes and producing visual sensations after interaction with the retina. The 'Biostack' experiments were designed with monolayers of biological objects sandwiched between nuclear track detectors, and demonstrated serious biological effects. The most arresting results with *Artemia salina* eggs showed that the hatching rate was reduced, that HZE particle collision with nauplius larvae caused abnormal growth and behaviour with a high mortality after hatching, stunted growth, delayed sexual maturity and reduced fertility. Advanced 'Biostack' experiments are designed for Spacelab to investigate this phenomenon which may pose a danger to non-regenerating human tissues particularly those of the central nervous system.

Space Shuttle

The reusable Space Shuttle will allow life scientists to work on orbit in a shirt sleeve environment for up to 30 days. The payload specialist will have a gentler ride than in the Saturn/Apollo system as now the maximum gravity load will be 3*G* and he will be content in the knowledge that a rescue capability is available. With less physical stress during flight and no direct flight responsibilities it is hoped that medical criteria for payload specialists will be much less strict than those applied to astronauts although this has not been the case with the initial selection.

A legacy from Project Apollo has been a feeling, reflected in government thinking, that spaceflight is remote from reality and too expensive. A policy of selecting life sciences payload specialists principally for their professional abilities may help to overcome this, improve the quality of research and in turn contribute to the safe, effective and extensive use of space. Both NASA and ESA are properly dependent on budget allocations from the member state governments and recent monetary restrictions may reduce the frequency of flights dedicated to the life sciences. This parsimonious attitude is perhaps surprising as astronomy, from the days of Stonehenge an interesting but costly diversion with little direct impact, is well funded. It is perhaps salutory to remind government agencies that space flight provides an important stimulus for diverse investigations with unpredictable results.

Shuttle flights will probably begin in 1980. The initial submission of life science experiments has been good and indicates considerable interest in the life science community. Finance for experiments may be more difficult but this system offers an opportunity to study the behaviour of biological systems in a new environment, and the applications are potentially unlimited. We should all now consider more carefully how we can use the unique properties of space to complement and enhance current research projects. □

Anthony W. Goode is a member of the Surgical Unit, The London Hospital.

REVIEW ARTICLE

Three-receptor, clonal expansion model for selection of self-recognition in the thymus

A. R. Williamson

Department of Biochemistry, University of Glasgow, UK

The postulate is made that dual recognition by T lymphocytes is due to two types of receptor, one encoded by antibody genes and one by a distinct multigene family with simple rules for expression. This postulate leads to a model explaining ontogenic and evolutionary selection for self recognition, T lymphocyte effector function, the apparent high frequency of alloreactive T cells and immune response gene activity. The model is contrasted with previous explanations of self-recognition phenomena.

ALL of the cell surface products coded for by genes of the major histocompatibility complex (MHC) have a crucial role in the regulation of the immune response. The cell surface products of the mouse H–2 complex have been divided into two classes: (1) those originally recognised as transplantation antigens (K and D); and (2) those recognised as immune associated (Ia) antigens (encoded at the H–2 I region). Initially I-region genes were shown to be important in regulating immune responses but more recently K- and D-region gene products have been shown to be involved in recognition and killing by T lymphocytes of cells that display foreign antigens on their surface. Thus cytotoxic T cells which have been generated in response to virus-infected cells[1], hapten-modified cells[2] or minor histocompatibility antigens[3] are only capable of lysing those target cells which bear the same foreign antigen and the same major histocompatibility antigens as the immunising cells. This phenomenon has been termed major histocompatibility restriction (thus for the mouse H–2 restriction) of cytotoxic T-cell responses.

Immune responses other than those involving cytotoxic T cells also show H–2 restriction. The proliferative response of T cells to antigen presented on the macrophage surface requires histocompatibility at the H–2 I region of the two cell types[4]. Similarly cooperation between helper T cells and B cells exhibits H–2 I restriction[5]; the fact that in chimaeric animals H–2 incompatible T and B cells are able to cooperate[6,7] being explained by the hypothesis that lymphocytes can adaptively differentiate, that is, develop the ability to recognise cell interaction molecules on allogeneic cells[8]. To explain the various types of H–2 restriction two types of hypothesis have been proposed[9].

The dual recognition hypothesis states that T cells exhibit two separate receptors on their surfaces, one of which shows specificity for H–2 antigens, the other for non H–2 antigens. In one form of this hypothesis the T-cell receptor for H–2 is itself an H–2 coded product whereas the receptor for foreign antigens is presumed to use the antibody gene repertoire[10]. In an alternative form of the hypothesis the two receptors are identical at an early stage in development and must therefore be encoded either by the H–2 linked gene system or by the antibody gene repertoire[11].

The altered self hypothesis postulates that T cells bear a single receptor which is only capable of recognising antigens in association with H–2, for example, new antigenic determinants created by the interaction of the foreign antigen with the H–2 antigen. In most models immunoglobulin variable region genes (V genes) have been assumed to code for the receptors.

Some form of dual recognition hypothesis is most attractive. The evidence for I-region restricted systems is more easily compatible with a dual recognition hypothesis than with an altered self hypothesis. Also, for the cytotoxic T cell, the dual recognition hypothesis has recently emerged as the favoured candidate. Indeed it has been argued forcibly that there is no viable form of altered self hypothesis[18].

In the initial demonstration of H–2 restriction the evidence was consistent with the idea that the immunising or target cells presenting the foreign antigen, needed to share histocompatibility antigens with the cytotoxic T cell. Thus restriction was seen as synonymous with a recognition of self antigens. Subsequently it was demonstrated that identity of histocompatibility antigens between immunising or target cell and the cytotoxic T cell is not necessary for recognition of self. The T cell 'learns' to recognise as self the histocompatibility antigens displayed on the epithelial cells of the thymus during the processing of T-cell precursors through that organ. This finding stems from experiments involving the use of bone marrow chimaeras and grafts of irradiated thymus tissue. Bevan[12] used chimaeric mice constructed by injecting (A × B) F_1 bone marrow into an irradiated parent A recipient to show that cytotoxic T cells generated against minor histocompatibility antigens recognise these antigens preferentially in association with the *H–2* haplotype of the parent A, as opposed to that of parent B. Zinkernagel *et al.*[13] constructed similar chimaeras and showed exclusive restriction for the H–2 type of parent A in cytolysis of either virally infected or hapten-modified target cells. Chimaeric mice constructed by injecting (A × B) F_1 bone marrow into irradiated (A × C) F_1 recipients were constructed and the lymphocytes sensitised against (B × C) F_1 cells chemically coupled to the hapten trinitrophenyl (TNP). In this case cytotoxicity was restricted to TNP-modified cells of the *C* haplotype. Thus T cells heterozygous for the haplotypes of A and B parents have learnt to recognise the H–2 type of the C mouse as self but failed to recognise the H–2 type of the B mouse (genotypically a self haplotype) as self. The use of irradiated thymus grafts in adult thymectomised irradiated recipients of bone marrow cells showed that the H–2 type of the thymus epithelium determines what the newly generated T cell learns to recognise as the self H–2 type[14].

Self recognition is clearly not determined by the identity of the H–2 haplotype between T cells and target cells. Zinkernagel *et al.*[13] pointed out that the learning of self recognition in the thymus is best explained on the basis of a dual recognition hypothesis because the altered self hypothesis would require the special rule that the specificities generated by modified self in thymus A do not overlap with the specificities generated in thymus B. However, the authors made no clear prediction as to whether dual recognition is achieved by two separate receptor molecules or two parts of a single receptor molecule.

These experiments and hypotheses had been influenced greatly by Jerne's prediction[15] of a role for the thymus in the selection of T-cell specificities. The model proposed by Jerne took as its starting point an inherited set of T-cell receptor antibodies each specific for an allelic form of one of the major transplantation antigens of the species, with the total repertoire reflecting the extent of polymorphism of the histocompatibility antigens of the species. The model then invoked rapid somatic

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mutation of the genes coding for receptor antibodies. Cells carrying receptors for non-self alloantigens were not eliminated in the thymus and emerged into the periphery in the high proportions expected for the products of germ line structural genes. These clones of cells explained the high frequency of allo-reactive T cells. T cells maturing in the thymus and expressing anti-self receptors were assumed to be eliminated unless those receptors mutated. In this way rapid somatic mutation was postulated to generate the total repertoire of receptors for all foreign antigens from the inherited genes for anti-self. This hypothesis was subjected by Bodmer[16] to the major criticism that individual members of a species could not be aware of the extent of polymorphism of the histocompatibility genes within the species. How then could there be a sufficient selective pressure for each animal to carry germ line genes coding for antibodies against the total repertoire of histocompatibility alleles? A second major criticism of the Jerne model for the generation of antibody diversity lay in the apparent difference in receptor repertoires between T and B lymphocytes. In particular it was not possible to detect the predicted high proportion of allo-reactivity at the B-cell level.

In spite of these major objections to the Jerne model it did predict the crucial selective role for the thymus that has been demonstrated recently. This has led Jerne and his colleagues[11] to propose a modification of the somatic mutation hypothesis for clonal selection within the thymus. Their modification involved two identical anti-self receptors, allowing mutation and selection to act on only one of them. This modification requires that special genetic mechanisms be invoked in addition to rapid somatic mutation. Other hypotheses explaining T-cell specificity in terms of V gene products also require rapid somatic mutation or rules of V gene expression unique to T cells[17–19]. I have therefore devised an alternative model for the selective function of the thymus and the reactivity of T lymphocytes.

Three-receptor, clonal expansion model

It is the main purpose of this article to propose a clonal selection hypothesis for the generation of T cells in the thymus that emphasises clonal expansion (rather than clonal deletion as in the Jerne model) and does not require somatic mutation.

The starting point for this model is the need to explain the basis for 'learning' of self from the phenotype of the thymus rather than from the genotype of the pre-T cell. It is proposed that the genotype of the pre-T cell must contain the information for a receptor molecule that can recognise allo-H–2 antigens as self and that this receptor is not an antibody molecule.

The model takes dual recognition as the basis for H–2 restriction and T-cell specificity and states that dual recognition is effected by two distinct receptor repertoires encoded by separate sets of genes. One type of T-cell receptor is encoded by immunoglobulin variable region genes and embraces the same repertoire and similar rules of expression as B-cell antibodies. The postulated second set of T-cell receptors is distinct from immunoglobulins, but has a repertoire of specificities similar to that originally described for immunoglobulin variable region *V* genes in the Jerne model[15] (but with contrasting consequences).

If pre-T cells of each genotype can recognise any thymus phenotype as self through an appropriate non-immunoglobulin receptor, then it follows that each animal carries genes coding for a repertoire of receptor molecules capable of recognising the repertoire of allo-antigenic specificities of major histocompatibility antigens. Thus receptors $R_1R_2R_3 \ldots R_n$ will recognise respectively the MHC antigens $H_1H_2H_3 \ldots H_n$.

The genes coding for these histocompatibility antigen receptors (R anti-H) are assumed to constitute a multigene family present in all members of the species. It is postulated that expression of genes in this family is limited to a single gene per haplotype in each cell, with no requirement for allelic exclusion. Expression of R anti-H occurs initially on pre-T cells. Each pre-T cell will express two R anti-H molecules selected at random from the inherited repertoire. A simple matrix of cell specificities is shown in Fig. 1. The combinations of R anti-H molecules on pre-T cells will be, in order of frequency: (1) both specific for allo-MHC antigens (R anti-allo-H); (2) one specific for a self-MHC antigen (R anti-self-H); (3) both specific for self MHC antigens. Examples of these three cell types in the matrix (Fig. 1) are: (1) R_2R_2; (2) R_1R_2; (3) R_1R_1.

Within the thymus the epithelium provides MHC antigens to interact with the R anti-H molecules on pre-T cells. The presence of at least one (or occasionally two) R anti-self-H molecules on a pre-T cell allows interaction with self-MHC antigens and this is postulated to give a proliferative stimulus to that pre-T cell (Fig. 2). Clonal expansion generates mature T cells all of which carry a receptor for a self-histocompatibility antigen. Because of the random diploid expression of R anti-H molecules the expanded population of T cells will be subdivided, in approximately *n* equal proportion, into *n* clones carrying either a second R anti-self-H or a single R anti-allo-H molecule. If we make the simple assumption that there are 100 R anti-H genes corresponding to 100 distinct allo-H specificities, then 1% of all T cells will carry one R anti-allo-H molecule corresponding to any particular allo-H specificity. The clonally expanded population of T cells will be exported from the thymus to the periphery. Pre-T cells which do not express at least one R anti-self-H molecule will not receive a proliferative signal leading to clonal expansion; such cells, if not eliminated, will be diluted out.

During the clonal expansion of pre-T cells driven by the interaction of R anti-self-H with self-H antigens each cell becomes committed to the expression of a particular receptor antibody molecule using the same repertoire of V genes encoding B-cell antibody molecules (Fig. 2). In B-cell development commitment of the pre-B cell to a single antibody specificity demands allelic exclusion and κ/λ exclusion. This exclusive commitment is probably effected by rearrangement of gene segments involving only two of the six V gene families present in the diploid cell[18–20]. For simplicity in the present model it is assumed that the differentiating T cell makes the same commitment as the differentiating B cell. Thus each T cell will express at random a single antibody V gene coded receptor molecule (whether both V_H and V_L contribute to this receptor is not important for the model). The entire repertoire of V gene encoded receptors will be expressed on individual T cells within each of the *n* expanding clones of pre-T cells defined by expression of pairs of R anti-H receptor molecules. It is necessary to invoke as a developmental limitation that when T cells become committed and express a V gene encoded receptor they cease being sensitive to the proliferative signal driving clonal expansion via the R anti-self-H receptor.

The postulated developmental limitation is simply explained by postulating an interaction between R anti-H and V anti-X. This interaction, or coupling, ensures that two signals are now required to stimulate the mature T cell. Because it is necessary to introduce a self tolerance selection acting on V anti-X this could be accommodated by the proposal that if V anti-X encounters X before the coupling of R anti-H and V anti-X the cell will be eliminated and tolerance will result. This tolerance model could involve dual recognition of X and self-H as proposed previously (ref. 23 and J. M. Phillips-Quagliata, personal communication).

Each mature T cell entering the periphery will carry three receptor molecules: (1) R anti-self-H; (2) R anti-allo-H ($100/n$% of the cells will carry second R anti-self-H); (3) a *V* gene encoded receptor antibody (Fig. 2). Restriction of responsiveness of T cells in the periphery will be accounted for if it is required that dual recognition involving R anti-self-H and a receptor antibody specific for the foreign antigen (X) is necessary for triggering an immune response. Restriction will be limited effectively to self-H specificities as determined by the R anti-self-H present on all T cells and selected in the thymus, but independent of the self-H type present on peripheral cells. A small subset of T cells will carry R anti-allo-H receptors capable of being involved in dual recognition of antigens presented together with allo-H specificities but, in the numerical example

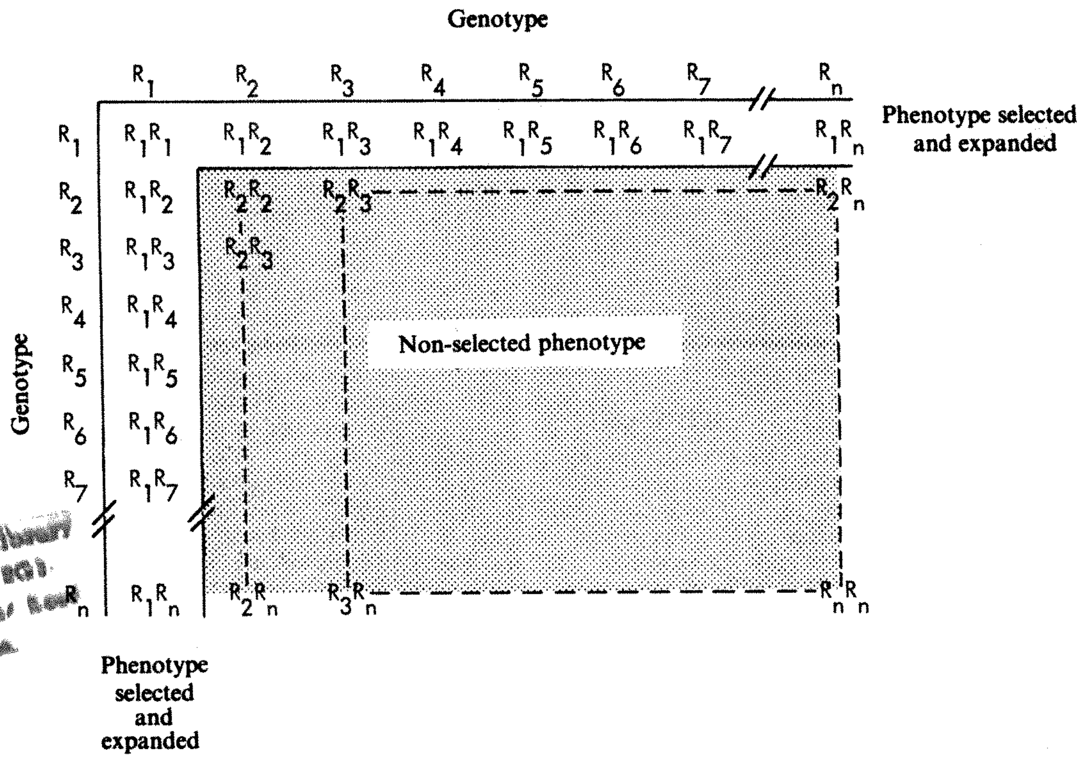
Genotype
R_1 R_2 R_3 R_4 R_5 R_6 R_7 R_n
Phenotype selected and expanded
R_1R_1 R_1R_2 R_1R_3 R_1R_4 R_1R_5 R_1R_6 R_1R_7 R_1R_n
R_2R_2 R_2R_3 R_2R_n
R_2R_3
Non-selected phenotype
Genotype
R_1 R_2 R_3 R_4 R_5 R_6 R_7 R_n
R_1R_n R_2R_n R_3R_n R_nR_n
Phenotype selected and expanded

Fig. 1 Cellular expression and selection of anti-histocompatibility receptor (R anti-H) genes.

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quoted above, such antigen presentation with allo-H specificities could only evoke a response at the 1% level relative to self-H presentation. However, in a situation where self presentation is not possible clonal expansion of this minor population of T cells could explain responsiveness[24].

Evolution of the R anti-H multigene family

One of the basic tenets of the present hypothesis is that each member of the species has the genetic capacity to make R anti-H molecules specific for the species repertoire of allo-H antigens. This parallels the specificity repertoire envisaged for germ line *V* genes in Jerne's hypothesis[15]. In that case, Bodmer[16] argued that it was almost impossible to conceive of a selective mechanism which could be powerful enough to maintain a situation requiring parallel evolution of histocompatibility antigens and immunoglobulin genes. Why then in the present model should a multigene family coding for R anti-species-H specificities be considered to be a feasible starting point. To answer this question we must look at the evidence which leads one to invoke such a multigene family and the selective pressures which could have led to the evolution of the gene family.

The unexpected ability of pre-T cells to 'learn' to recognise as self the phenotype of the thymus epithelium, even when this phenotype is at variance with the genotype of the pre-T cells, leads to the logical postulate that the genotype of one strain codes for receptor molecules able to recognise histocompatibility antigens of another strain. The limitations to this allo-recognition process can only be uncovered by further experimentation. The evidence also indicates that it is essential for immune responsiveness that each animal exhibits complementary R anti-H and H molecules[14]. If the genes coding for R anti-H and H are segregating independently and the H antigens are highly polymorphic there will be a selective pressure for the accumulation in the R anti-H gene family of genes coding for all possible R anti-H specificities. Genes coding for a complete (or extensive) repertoire of R anti-H specificities will be maintained in the germ line of the species because of the strong selective pressure for each member of the species to be able to recognise its own histocompatibility antigens as self. It is important to this argument that gaps in the immune responsiveness of T cells due to an absence of certain R anti-self-H genes in a given member of the species will lead to an increased susceptibility to viral infection and therefore an increased probability of the elimination of that individual before reproduction. There will be a strong selective pressure, enhanced by any polymorphism of the R anti-H genes, for MHC heterozygosity to maximise the chance that self can be recognised.

The multigene family coding for R anti-species-H specificities is postulated to be distinct from the immunoglobulin gene families and is not postulated to be encoded at the MHC as previously invoked for the T-cell receptor. However, it is important to realise that the expression of particular R anti-self-H genes will be controlled by the MHC so that the R anti-H T cell receptor will appear to be encoded at the MHC.

It might be expected, on the basis of the known specificity of restriction, that each R anti-H recognises a private histocompatibility antigen. This prediction explains the finding[25] that a private (and not a public) H–2 specificity is recognised in the restriction of a cytotoxic response to virus infected cells although public H–2 specificities are recognised in anti-allo-H–2 responses, both cytotoxic and proliferative. In both responses the private H–2 antigen is postulated by the present model to interact with R anti-H. In the anti-viral response the V receptors will recognise viral antigens while in the anti-allo-H–2 response the V receptors will be specific for both private and public H–2 antigens. It should be possible to define the repertoire of R anti-H specificities from the restriction of responses by private H–2 specificities.

T-cell effector types

The hypothesis automatically accounts for the finding that separate populations of T cells recognise antigen in association with each histocompatibility antigen (for example, in the mouse T cells are specific for either K, D or I antigens). The model is consistent with the division of T cells into subsets having different effector functions but each displaying a full repertoire of specificities[26,27]. The expression of R anti-K or R anti-D will lead to T cells destined to become cytotoxic cells, while the expression of R anti-I will lead to T cells destined to become helper cells. The model predicts that individual clones of cytotoxic T cells will show either K or D restriction[28–30]. This specificity will be provided without the need to invoke separately controlled repertoires of genes coding for R anti-K, R anti-D and R anti-I. A single multigene family containing an assortment of genes coding for each of these receptor types will, on the basis of the random expression of one receptor per haploid set per cell, infrequently give rise to a cell expressing two receptors with specificities for antigens of the same haplotypes. Most cells will display one R anti-self-H together with one R anti-non-self-H. Allelic exclusion of R anti-H expression is not necessary for clonal restriction to K, D or I recognition.

The small percentage of T cells displaying two different R anti-self-H specificities would not be expected to make a significant contribution to immune responsiveness, which will therefore be effectively clonally restricted for a single H specificity (K, D or I). However, the small fraction of T-cell clones with any given third receptor for R anti-allo-H will be able to recognise the foreign antigen in association with that allo-H specificity. Such a subset of T-cell clones has been found in limiting dilution experiments[31].

Frequency of allo-H-2 reactive cells

One of the major problems which any model for T-cell receptors and self recognition must explain is that of the high frequency of cells showing specificity for allo-histocompatibility antigens[32-34]. The present clonal expansion model contains an inherent explanation for this phenomenon. Before this explanation is discussed, the nature of the problem should be clarified. There may well be both a qualitative and a quantitative difference in the perception by T cells of H-2 antigens relative to all other antigens. The absolute frequency of allo-H-2 reactive cells is clearly high but is a difficult figure to establish with any accuracy. Frequently allo-H-2 reactive cell frequencies in excess of 1%[35-38] are compared with frequencies of $10^{-6}-10^{-5}$ for cells responsive to conventional antigens (the latter figure appears to be drawn from data on the specificities of B cells). When precursor cell frequencies for allo-H-2 reactive cytotoxic T cells determined in a limiting dilution assay were compared with the frequency of cytotoxic cells specific for a hapten (TNP) the frequency of hapten responsive cells was of the order of tenfold (between eight- and thirty-fold) less than the frequency of alloreactive cells[37]. Moreover when the allo-H-2 difference was restricted to either the K or D end of H-2, the frequencies of reactive cells were comparable with those specific for TNP. It is therefore possible that the major difference in frequency of antigen specific cells is between the B-cell and T-cell populations rather than between the frequency of allo-H-2 reactive T cells and T cells of all other specificities. In this regard it is noticeable that B-cell responses to H-2 allo-antigens are much less pronounced than corresponding T-cell responses.

With the above discussion in mind, let us look at the receptor specificities predicted by the present model to be present on cells reactive with allo-H-2 antigens or other foreign antigens (designated X). T cells reactive to X must display R anti-self-H (K, D or I) together with a V receptor specific for X. The V receptor repertoire on B cells and T cells is identical but it is reasonable to expect that with dual recognition there will be activation of T cells possessing V receptors with lower affinity for X than is necessary for activation of a B cell. This difference in affinity threshold could account in part for the apparently higher frequency of T cells reactive with X relative to B cells reactive with X. T cells reactive with allo-H-2 differences will need to possess R anti-allo-H (K, D or I) together with V receptors specific for H-2 allo-antigen differences. In the case of responses limited to differences at K or D loci the frequency of reactive cells would not be expected to be higher than the frequency of cells reactive to X. However, many measurements of alloreactivity have been made using strains of animals different at multiple loci (major and minor histocompatibility differences). In such cases a much bigger proportion of T cells bearing R-anti-allo-H molecules could be activated because the repertoire of V receptors specific for all other antigenic differences would be susceptible to stimulation in a dual recognition manner.

Passive selection systems involving the absorption of T cells by exposure to monolayers of histoincompatible cells[39] or by passage through histoincompatible animals[40] have been used to assess the fraction of T cells binding a particular antigen. In posing the problem of the proportion of allo-H-2 reactive cells the frequencies determined in these passive selection assays should be clearly distinguished from the frequencies determined in assays for precursors of effector cells. It should be remembered that in the B-cell system the frequency of antigen-binding cells is often found to be 10- to 100-fold greater than the frequency of B cells acting as precursors for specific antibody-producing cells[41]. It is reasonable to assume that V receptors behave similarly on both B and T cells and that a difference in frequency between antigen-binding cells and antigen reactive cells might be seen for T cells as it has been for B cells. In the present model this would imply that a considerable proportion of T cells carrying R anti-allo-H-2 might be removed in passive selection. In addition cross reactivities at the level of R anti-H receptors, not detectable at the level of responding cells, might be revealed by passive selection methods.

The percentage of cells responding in allo-aggression has led to the suggestion, and to evidence supporting that suggestion[32,42], that T cells are not monospecific as required for clonal selection. In the present model the T cells responding in allo-aggression are monospecific (monoclonal) with respect to R anti-allo-H specificity but that population comprises a multiplicity of T cells each monospecific with regard to its V region coded receptor and each expanding in an antigen-driven monoclonal fashion.

In addition to containing an inherent explanation of alloreactivity the present model appears to offer explanations for a number of other phenomena.

T-cell receptor idiotypes and antibodies to T-cell receptors

Considerable evidence is accumulating to show that T cells specific for either foreign antigens or alloantigens have receptor molecules carrying idiotypes identical to B-cell antibodies specific for the same antigens[43,44]. It is axiomatic to the present hypothesis that these idiotypes are encoded by the same genes.

The presence of identical idiotypes on B and T cells allows for the physiological action of anti-idiotypic antibody on both sorts of cells postulated in an idiotypic network hypothesis[45]. It is reasonable to suppose that the R anti-H family of molecules, unlike the V encoded idiotypes, will not be included in the network. Therefore, the T cell would be susceptible to anti-idiotypic network signals only through its allelicly excluded *V*-gene encoded receptor. One exception to the latter postulate might occur in allogeneic responses. Immunisation of an animal with allogeneic cells (MHC differences) will result in production of antibody to the MHC antigens. An antibody response to the R anti-H molecules on the antigenic cells could also occur. Antibody specific for R anti-H molecules might be expected to act like antibody against the idiotype of anti-MHC antibodies[46] and therefore might regulate the production of antibody against MHC antigens.

The reverse situation, that anti-idiotypic antibodies specific for the V encoded idiotypes cross react with R anti-H molecules, is also possible and might account for those data indicating a high percentage of T cells bearing allo-MHC specific idiotypes[47]. Note that in other studies such a high percentage of idiotype-bearing T cells has not been found[48].

The possibility, engendered by the present model, that antibodies might be raised against R anti-H in an immunisation regimen designed to raise antibodies against MHC antigens, has implications for the use of such antisera in functional assays. Blocking of T-cell function attributed to anti-MHC antibodies could be due to the presence of anti- (R anti-H) antibodies. Monoclonal anti-MHC antibodies will not be subject to this problem.

Function of T-cell receptors: responses involving K and D regions

This presentation is concerned mainly with recognition processes but must also relate the function of T-cell receptors. The

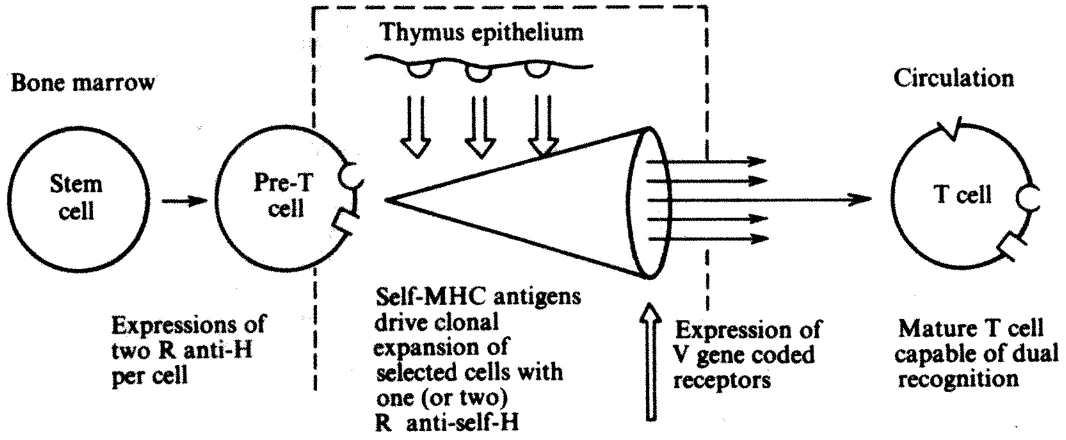
Thymus epithelium
Bone marrow
Circulation
Stem cell
Pre-T cell
T cell
Expressions of two R anti-H per cell
Self-MHC antigens drive clonal expansion of selected cells with one (or two) R anti-self-H
Expression of V gene coded receptors
Mature T cell capable of dual recognition

Fig. 2 Stages in T-lymphocyte maturation.

functional aspects of dual recognition have been discussed extensively by Langman[49] and by Cohn and Epstein[18]. The fact that the receptors in the present model arise in part from genes not previously invoked, and without the need for the somatic mutation and selection necessary in previous models does not alter the functional arguments. The requirement for a concerted response to X and an MHC antigen can be met either by interaction between X and MHC[19] or by interaction between the receptors for X and MHC[18]. In the present model an interaction between the two types of receptor occurs and is necessarily selected for during development. This interaction prevents stimulation of the R anti-H receptor. Binding of antigen to V anti-X will make R anti-H available to bind H present on the same cell surface as X. When R anti-H binds H, this completes the stimulatory complex initiating the precursor cell to proliferate and differentiate and triggering the cytotoxic cell to kill. The stimulating signals can be delivered to the cell via either R anti-H alone or R anti-H plus V anti-X; this is true in the present model for all interactions, whether self presentation or allogeneic responses, because an R anti-H molecule is always involved. There is no need to postulate (as in other models[18]) either that no signal can be delivered by the R anti-H receptor or that allogeneic reactions do not involve the R anti-H receptor.

It is necessary to postulate that interaction of V anti-X with X while the R anti-H remains unsatisfied will not deliver a signal to the T cell. Indeed the requirement for both receptors to be filled for passive absorption of cytotoxic T cells[50] suggests that the V anti-X interaction with X may be transient.

Functions of T-cell receptors: responses involving the I region

A special role has been demonstrated for the I-region products in the generation and function of helper cells for antibody production[4,5] or in mixed lymphocyte reactions (MLR)[51]. However, MLR stimulation can be demonstrated between cells matched at the MHC[52] or differing at K or D antigens but matched for the I region; the extreme examples are the H-2 mutants which appear to be point mutations of either the K or D products[53]. In the present model all MLR stimulation will involve dual recognition. In a fully allogeneic MLR, including antigenic differences at I-region gene products, dual recognition will involve an R anti-allo-I molecule and a V gene encoded receptor specific for any antigenic difference. With a minimal difference the active cells in MLR between parent strain and H-2 mutant strain would be T cells carrying R anti-self-I together with V receptors specific for the mutant histocompatibility antigens.

A valid model of T-cell function should explain MHC-linked immune response (*Ir*) genes[54]. These *Ir* genes regulate the production of antibody. An *Ir* genotype is classified as allowing either high or low responsiveness; the low responder phenotype exhibits a lack of functional helper T cells specific for the antigen under *Ir* gene control (but low responders do have T cells capable of binding that antigen[55]). Inheritance of *Ir* genes has been interpreted as showing either dominance of the high responder gene[54] or co-dominance[56].

A recurrent explanation[18,19,54] offered for *Ir* gene function is that *Ir* genes encode the T-cell receptor. Such explanations postulate that the structural genes for the T-cell receptor map in the I region, thus contradicting evidence that the T-cell receptor is encoded by structural genes linked to immunoglobulin heavy chain genes[43] (although there is evidence for genes at the MHC controlling T-cell receptor expression[48]). Two contrasting explanations of the non-responder phenotype have been offered in terms of the somatic mutability of V genes. In one model[15] all V anti-X genes are generated by mutation of V anti-self genes but in the Ir low responder a V gene specific for the antigen cannot be generated from any anti-self V gene. In another model[18,49] it is postulated that only an anti-self V gene can generate a V gene coding for a product specific for the antigen under *Ir* gene control and low response is due to the need to retain V anti-self unmutated to perform its function in dual recognition. In neither of these two models is it obvious why *Ir* genes controlling antibody production map in the I region rather than in K and D regions of the murine MHC. A different explanation for Ir defects describes *Ir* gene products as a second recognition system with non-responsiveness resulting when the *Ir* gene product cannot associate effectively with an antigen[19]. The explanation for MHC-linked *Ir* genes in the present model attributes non-responsiveness to the binding of the antigen to the R anti-I specific for the non-responder I-region product. The functional *Ir* gene is thus one of the T-cell recepors; it is not encoded at a locus linked either to an immunoglobulin gene family or to MHC but expression of *Ir* gene phenotype will be controlled by I-region products so that the MHC appears to encode *Ir* genes.

Complete humoral responsiveness to every antigen is postulated to involve the R anti-I T-cell receptor but in most cases Ir control is not seen because low responsiveness is displayed only when an antigen fortuitously binds to the R anti-I, thus blocking the interaction of R anti-I with I in the dual recognition process. This defect will be seen at the level of antigen presentation on macrophages and at the level of co-operation between T and B lymphocytes because both processes involve simultaneous recognition of I region and antigen. The postulate that Ir phenotype coincides with R anti-I specificity leads automatically to an explanation for the findings that pre-T cells with a non-responder phenotype, when processed by a thymus of responder genotype, emerge as responder T cells[57] because there will have been selection for R anti-responder-I specificity. In keeping with the evidence[57], the model says that T cells generated thus will only function as responder helper cells when complementing responder B cells and macrophages. Because this model requires that all R anti-self-I molecules are selected for in the thymus it follows that *Ir* genes should show co-dominant expression[56].

Predictions

The model proposed here predicts that each T cell carries three receptor molecules. Two of these are non-antibody receptors, at least one of which is specific for a self-MHC molecule (either K, D or I in the mouse). Detection and characterisation of R anti-H molecules would provide evidence for the model. A small fraction of T-cell clones responding to a foreign antigen recognised together with a self-H antigen will also be able to recognise the antigen presented with an allo-H antigen due to the postulated third receptor (R anti-allo-H). Evidence for the latter subset of T-cell clones has been published[31].

Receptors specific for self-MHC are postulated to be the products of a multigene family encoding receptors for many allo-MHC products. The extent of that gene family will be revealed by further tests of the ability of pre-T cells to be selected in the thymus for recognition of a range of MHC types as self. Because expression of receptors for self-MHC molecules is postulated to be selected by the MHC antigens of the thymus the MHC appears to code for the T-cell receptor. An involvement of anti-self-MHC receptors in I-region linked non-responsiveness is postulated.

While recognising that I have invoked a new gene family that may or may not exist, I trust that the concept has some merit in ordering existing data and that it may influence future experimental design.

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ARTICLES

Observations of flexure and the geological evolution of the Pacific Ocean basin

A. B. Watts & J. H. Bodine
Lamont-Doherty Geological Observatory, and Department of Geological Sciences, Columbia University, Palisades, New York 10964
N. M. Ribe
Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

The response of the Pacific plate to oceanic island and seamount loads has been used to estimate the distribution of ridge crest and off-ridge volcanism on the plate since the late Jurassic. The most extensive event, forming the Hess rise, Line Islands ridge, Necker ridge, and Robbie ridge, occurred on the Pacific/Farallon and Pacific/Phoenix ridge crests during the interval 90–120 Myr BP *(Barremian to Turonian). This event, which extended over a much larger area than comparable volcanism on the continents, is one of the largest to be documented in the geological record.*

THE Pacific Ocean floor is characterised by numerous oceanic islands and seamounts of mainly volcanic origin[1-4]. Although the age of the oceanic crust of the Pacific basin is now reasonably well known from marine magnetic lineations and Deep Sea Drilling Project (DSDP) sites, the origin and age of these volcanic features are largely unknown. These oceanic islands and seamounts are of geological interest as they are located within the interior of the Pacific plate and may therefore provide information on intra-plate volcanism. This article presents a method, based on oceanic plate flexure, for determining the origin and age of some of these volcanic features in the Pacific Ocean basin.

Lithospheric flexure

The concept of plate tectonics is based on a strong rigid lithosphere which overlies a weaker asthenosphere. The principal evidence for a strong lithosphere on long time scales ($>10^6$ yr) has come from studies of its response to surface loads[5-15]. These studies have shown that in several cases, geological and geo-

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physical data in the region of surface loads can be explained by a simple model in which the lithosphere responds to long-term loads in a manner similar to that of a thin elastic plate overlying a weak fluid.

The results of recent studies of oceanic plate flexure are summarised in Fig. 1 in which the elastic thickness of the oceanic lithosphere T_e is plotted as a function of the age of the lithosphere at the time of loading. Different types of geological features have been plotted in Fig. 1. The age of the lithosphere at the time of loading was estimated at each feature by subtracting the age of the load from the age of the underlying sea floor. The studies by Watts[9] and McNutt and Menard[10] are shown as dashed lines because a single estimate of T_e was obtained for a wide range of age at the time of loading (>60 Myr). Also shown as a dashed line is the study by Caldwell[14] at the Aleutian trench as it is difficult to separate the Aleutian outer rise from a broad regional rise in bathymetry which occurs seawards of the trench[16]. Figure 1 summarises all recent estimates of T_e, except those from the Philippine and Tonga–Kermadec trenches[14] where the age of the subducting oceanic crust is too poorly known.

The main results shown in Fig. 1 are that surface loads formed on young lithosphere, such as the topography of oceanic Layer 2, are associated with relatively small values of T_e while loads formed on old lithosphere, such as the Hawaiian–Emperor seamount chain, are associated with relatively high values. The simplest interpretation of these results is that as the oceanic lithosphere increases in age it cools, thickens, and becomes more rigid in its response to surface loads. Figure 1 shows that there is good general agreement between T_e and the 300 to 600 °C oceanic isotherms, based on a cooling plate model[17].

As the age of the surface loads plotted in Fig. 1 are in the range of a few Myr to several tens of Myr, Fig. 1 suggests T_e is acquired at the time of loading and does not change appreciably with time. This result is consistent with thermal models which predict that the thickness of the lithosphere increases with age[9] and with inferences of the rheology of the oceanic lithosphere based on extrapolations of data from experimental rock mechanics[18,19].

The results in Fig. 1 have important implications for studies of the geological evolution of the ocean basins. For example, Fig. 1 suggests that an oceanic island or seamount formed on young sea floor would be associated with a relatively small T_e, while an oceanic island or seamount formed on old sea floor would be associated with a relatively large T_e. Thus by determining T_e at an oceanic island or seamount of unknown origin it should be possible to estimate whether it formed on young (ridge crest) or old sea floor (off-ridge).

A geophysical observation which can be relatively easily measured on a surface-ship and depends on T_e is the free-air gravity anomaly[7–9,12]. For the theoretical seamount load shown in Fig. 2 there is a significant difference in both amplitude and wavelength between computed gravity anomaly profiles for $T_e = 5$ km, corresponding (Fig. 1) to formation of the load on relatively young sea floor (2–8 Myr), and $T_e = 25$ km, corresponding to formation on relatively old sea floor (>35 Myr). Thus since the overall accuracy of gravity data in the Pacific Ocean is 5–10 mGal (ref. 16), Fig. 2 suggests it should be possible to use observed free-air gravity anomaly profiles to distinguish whether a geological feature originated on a ridge crest or off-ridge.

Data analysis

We have compared observed free-air gravity anomaly profiles obtained on more than 100 cruises of mainly US and USSR research vessels in the Pacific Ocean to calculated profiles based on the elastic plate model. We assumed values of $T_e = 5$ and 25 km since these values clearly represent a ridge crest or off-ridge origin respectively (Fig. 1). The calculated profiles were obtained using linear transfer function techniques[7,9] in which theoretical filters are constructed and convolved with observed bathymetry profiles. Both two- and three-dimensional filters[7] and the effects of non-linearity in the computation of the gravity anomaly have been considered.

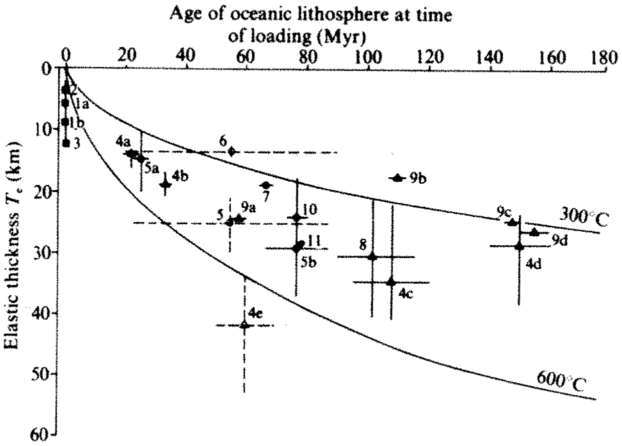

Fig. 1 Plot of elastic thickness of the oceanic lithosphere T_e against age of the lithosphere at the time of loading. 1a, East Pacific rise crest[5]; 1b, Mid-Atlantic Ridge crest[5]; 2, Juan da Fuca ridge crest[6]; 3, Mid-Atlantic Ridge crest[7]; 4, Deep-sea trench outer rise systems[14]; 4a, Nankai trough; 4b, Middle America trench; 4c, Kuril trench; 4d, Mariana trench, 4e, Aleutian trench; 5, Hawaiian–Emperor seamount chain[9]; 5a, Emperor seamounts north of 40° N; 5b, Hawaiian ridge and Emperor seamounts south of 40° N; 6, Pacific Ocean atolls[10] (Cook Islands, Australs, Tuamotu Islands and Henderson); 7, Great Meteor seamount, NE Atlantic[8]; 8, Kuril trench[15]; 9, Deep sea trench-outer rise systems[13]; 9a, Aleutian trench; 9b, Kuril trench; 9c, Bonin trench; 9d, Mariana trench; 10, Hawaiian ridge[11]; 11, Hawaii Island[12]. Each estimate of T_e is related to the flexural rigidity of the plate and is based on an assumed Young's modulus of 1×10^{12} dyn cm^{-2}. The age of the lithosphere at the time of loading was estimated by subtracting the age of each load from the age of the underlying sea floor. For the ridge crest estimates and trench estimates the loading is assumed to be of the present age (0 Myr). For the seamount and oceanic island estimates, the age of loading is obtained from dated samples on the crests or flanks of these features. The solid lines are the 300 °C and 600 °C oceanic isotherms based on a cooling plate model[17].

Figure 3 shows the comparison between observed and computed gravity anomaly profiles for four selected geological features in the Pacific Ocean. The observed gravity anomaly profiles of the Line Islands ridge and Mid-Pacific mountains can be most satisfactorily explained by the computed profile with $T_e = 5$ km (Fig. 3*a*). The computed profile with $T_e = 25$ km predicts too large an amplitude gravity anomaly over the crest of these features compared with the observed amplitude and wavelengths that are too long over flanking regions. The observed gravity anomaly profiles of Makarov Guyot[3] and Louisville ridge, however, can be most satisfactorily explained by the computed profile with $T_e = 25$ km (Fig. 3*b*). The computed profile with $T_e = 5$ km predicts too small an amplitude over the crest of these features compared with the observed amplitude and wavelengths that are too short over flanking regions.

The comparisons in Fig. 3 can therefore be interpreted as indicating the Line islands ridge and Mid-Pacific mountains formed on a ridge crest (on sea floor of 2–8 Myr age) while Makarov Guyot and Louisville ridge formed off-ridge (on sea floor of >35 Myr age). These conclusions on the origin of these features are, of course, uncertain as they are based on a single gravity anomaly and bathymetry profile and refer only to the origin of the major part of each feature. We have shown[21], however, that uncertainties in the computed profiles, due either to variations in the crustal structure and density of topography assumed or to non-linearity in the computed gravity effects would not significantly alter these conclusions.

Results

The results of the data analysis for each of the cruises in the Pacific Ocean are summarised in Fig. 4: the symbols represent an estimate of the geological setting of an individual bathymetric feature along each ship track. Figure 4*a* summarises those

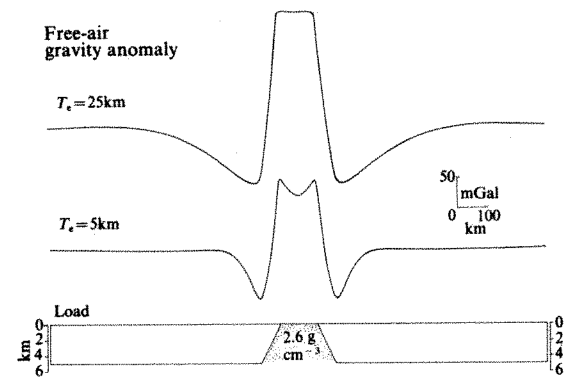

Fig. 2 Computed free-air gravity anomaly profiles based on the continuous elastic plate model and the theoretical seamount load shown. The flexure of the plate has been computed using linear theory[20] and the free-air gravity anomaly has been computed using a two-dimensional line-integral method.

estimates which are interpreted to be of ridge crest origin and Fig. 4*b* those estimates which are of off-ridge origin.

A comparison of Fig. 4*a* with available bathymetry maps of the Pacific Ocean[24,25] show that most of the ridge crest estimates correlate with bathymetric features in the western and central Pacific. These include the Manihiki plateau, Shatsky rise, Hess rise, Necker ridge, Line Islands ridge and Tuamotu Islands and some features in the Magellan seamounts, Mid-Pacific mountains and Emperor seamounts. A number of these features were associated with more than two ridge crest estimates (for example, Shatsky rise (5), Necker ridge (3), Hess rise (4), Line Islands ridge (5) and Tuamotu Islands (10)). Most of the ridge crest estimates (Fig. 4*a*) apparently occur between the 90 and 120 Myr isochrons, based on magnetic anomaly lineations and DSDP sites[22].

The off-ridge estimates (Fig. 4*b*) mainly correlate with bathymetric features in the western Pacific Ocean. These include Society Islands, Samoa, Melanesian border plateau[23], Cross-Line Island trend and Louisville ridge and some features of the Hawaiian ridge and Emperor seamounts, Geisha and Marcus–Wake Guyots[3] and Magellan seamounts. A number of these features were associated with more than two off-ridge estimates (for example, Samoa (3), unnamed seamount in the Magellan seamounts (3), Louisville ridge (3), and Society Islands (3)). The off-ridge estimates show no obvious relationship to the isochrons although the estimates for the Cross-Line Island trend, Hawaiian ridge and Marcus–Wake Guyots correlate with bathymetric features which generally trend WNW–ESE[4], across the local trend of the isochrons.

We recognise that although data from a large number of cruises have been used in this study, the number of estimates in Fig. 4 is small compared with the total number of oceanic islands and seamounts which actually exist in the Pacific Ocean. We believe, however, that although any one estimate in Fig. 4 may be subject to uncertainties, the occurrence of several similar estimates from the same geological feature is evidence that the overall distribution of ridge crest and off-ridge estimates (Fig. 4) is correct. A better test of their reliability, however, is to compare the predicted age of the bathymetric features associated with these estimates to geological ages based on bottom sampling.

The ages of the bathymetric features associated with each estimate (Fig. 4) can be predicted if the age of the underlying sea floor is known (Fig. 1). Table 1 summarises those estimates which are associated with a nearby reliably dated bottom sample. There is a good general agreement between predicted and geological ages for the ridge crest estimates, particularly for Hodgkins, Line Islands, Easter Island and the Tuamotu Islands. The agreement is not as good for the off-ridge estimates because the predicted ages are only maximum estimates.

Table 1 therefore suggests that it should be possible, at least for the ridge crest estimates (Fig. 4*a*), to predict the age of the associated bathymetric features with some confidence. The main uncertainties, however, are in estimating the age of the sea floor, particularly in regions of the magnetic quiet zones. We tabulated the predicted ages for each ridge crest estimate in 30-Myr intervals through geological time. The smallest number of estimates (3) occur for the interval 150–180 Myr BP (Kimmeridgian to Pliensbachian) and the largest number of estimates (26) occur for the interval 90–120 Myr BP (Barremian to Turonian).

The distribution of the off-ridge and ridge crest estimates in the Pacific Ocean basin for the 90–120 Myr BP interval are plotted in Fig. 5. The solid triangles represent ridge crest estimates associated with the Hess rise, Necker ridge, Line Islands ridge, Manihiki plateau and Robbie ridge and the solid circles represent off-ridge estimates associated with the Geisha Guyots, for which there is geological control (Table 1). The bathymetric features associated with the ridge crest estimates extend over a broad region of the Pacific Ocean basin (Fig. 5). We therefore infer that the interval 90–120 Myr BP was a time of significant volcanism on the Pacific plate.

As the solid triangles in Fig. 5 represent ridge crest estimates they may indicate the former position of the accreting Pacific

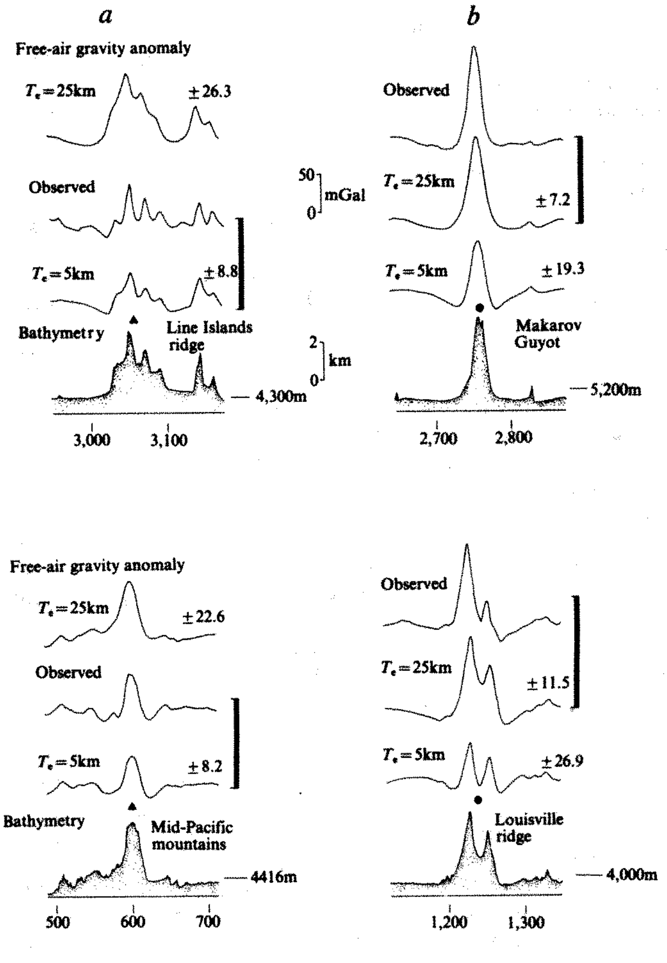

Fig. 3 Comparision of observed free-air gravity anomaly profiles with computed profiles based on the elastic plate model and assumed values of the elastic thickness $T_e = 5$ km and 25 km. *a*, USNS Eltanin 31 profile of the Line Islands ridge at lat 1.7° N and long 157.0 °W and RV Vema 24 profile of the Mid-Pacific mountains at lat 18.5° N and long 179 5° W. *b*, RV Vema 21 profile of Makarov Guyot at lat 29.6° N and long 153.5° E and USNS Eltanin 40 profile of Louisville ridge at lat 39.3° S and long 167.5° W. The observed profiles of the Line Islands ridge and Mid-Pacific mountains can be best explained by the computed profile of $T_e = 5$ km and the observed profiles of Makarov Guyot and Louisville ridge can be best explained by the profile for $T_e = 25$ km. A ridge crest origin (▲) has been assigned to the Line Islands ridge and Mid-Pacific mountains; an off-ridge origin (●) has been assigned to Makarov Guyot and Louisville ridge. The numbers to the right of each computed profile are the r.m.s. differences between observed and calculated gravity anomalies and the number below each bathymetry profile is the distance in nautical miles along a ship track, used to locate each feature.

Table 1 Comparison of predicted ages based on flexure studies with geological ages

Seamount or oceanic island	Ship crossing feature	Position of ship crossing		Method of geological age determination	Position of geological age		Estimated age of underlying sea floor (Myr BP)	Predicted (flexure) age* (Myr BP)	Geological age (Myr BP)	Geological age ref.
		Lat	Long		Lat	Long				
Ridge Crest										
Hodgkins (Kodiak–Bowie)	Conrad	53.3	−135.6	K–Ar	53.5	−136.0	17	12 ± 3	14–15	26
Necker ridge	Vema	22.5	−166.7	K–Ar	21.5	−167.9	100	95 ± 3	>61	27
Line Islands	Kana Keoki	3.8	−159.4	DSDP Site 315	4.2	−158.5	102	97 ± 3	91 ± 3	28
Shepard Guyot	Vema	18.5	−179.5	Forams	19.2	−179.5	137	132 ± 3	≥Cen–Tur.	3
Manihiki plateau	Kana Keoki	−12.1	−160.9	DSDP Site 317	−11.0	−162.0	?90	?85 ± 3	≥105	28
Shatsky rise	Conrad	32.1	155.1	DSDP Sites 305, 306	32.0	157.8	136	131 ± 3	≥120	29
Easter Island	Conrad	−27.2	−109.5	K–Ar	Samples from island		4	<2	3	29
Tuamotu	Conrad	−15.6	−147.4	DSDP Site 318	−14.8	−146.8	61	56 ± 3	49–53	30
Wentworth	Kana Keoki	28.8	−177.9	K–Ar	28.8	−177.8	110	105 ± 3	≥71	31
Off-ridge										
Isakov Guyot	Vema	31.6	151.1	Fossil	31.6	151.2	148	<113	≥Lower cretaceous	3
Makarov Guyot	Vema	29.5	153.5	Fossil	29.5	153.3	155	<120	≥Cen–Tur.	3
Z4/3 Guyot†	Vema	27.1	148.7	K–Ar	27.1	148.7	154	<120	≥86–96	32
Lamont Guyot	Argo	21.7	159.4	Fossil	21.5	159.2	170	<135	≥Mid–Eocene	3
Ita Mai Tai Guyot	Dmitri Mendeleyev	12.9	157.0	DSDP sites 200, 202	12.8	156.8	171	<136	≥Early Eocene	33
Somoa (Savaii)	Dmitri Mendeleyev	−13.3	−172.3	Historic eruption	−13.6	−172.4	107	<72	Historic eruption	34
Society	Kana Keoki	−16.1	−151.3	K–Ar	−16.7	−151.0	71	<36	1.9–5.4	35

* Computed assuming a ridge crest estimate corresponds to an age of the lithosphere at the time of loading of 2–8 Myr and an off-ridge estimate corresponds to an age of >35 Myr.

† This seamount has a reliable palaeomagnetic pole[49]. The new constraints on the age suggests this pole (lat. 53.0° N, long. 302°) is probably of early Cretaceous age.

plate boundary. In particular, the Line Islands ridge, Necker ridge and Hess rise probably formed close to the crest of the former Pacific/Farallon plate boundary[36] and the Robbie ridge probably formed close to the crest of the former Pacific/Phoenix plate boundary[36]. Therefore, either volcanism occurred along a major portion of the Pacific plate boundary during 90–120 Myr BP or, beginning about 90–100 Myr BP major ridge jumps occurred along the Pacific plate boundary[4] accompanied by extensive volcanism on the abandoned ridges. Because of the scatter of the ridge crest estimates (Fig. 5) and the fact that only one-limb of the 90–120 Myr BP spreading system is preserved at present, it is not possible to distinguish between these possibilities.

Recent evidence from DSDP site 462[37] and from seismic reflection profiling with a large volume sound source[38] support the earlier suggestion of Winterer[4] that the relatively old crust of the central Pacific basin and the Line Islands area may be obscured by a younger deep-water volcanic event. DSDP site 462[37] (Fig. 5), drilled on oceanic crust of probable late Jurassic age, recovered a volcanic igneous complex of Albian to Aptian age (100–110 Myr BP). Houtz and Ludwig[38] have mapped a seismic reverberant layer, using sonobuoy and single channel vertical incidence data, which reaches thicknesses exceeding 2 km in parts of the central Pacific Ocean basin. Although the reverberant layer may be of calcareous origin[38], the presence of a thick layer (>600 m) in the region of DSDP sites 462 and 169 suggests the layer may be, in part, of volcanic origin and therefore similar in age to the volcanic rocks drilled at the DSDP sites.

These considerations suggest the 90–120 Myr BP ridge crest volcanism which formed the Line Islands ridge, Hess rise and Necker ridge (Fig. 5) may have been accompanied by an extensive deep-water volcanic event in the adjacent ocean basins. The solid line in Fig. 5 outlines the area of the Pacific plate which seems to have been most affected by this event. We have included only evidence from the ridge crest estimates (Fig. 4*a*), the off-ridge estimates for which there is geological control (Table 1) and the contemporaneous deep-water volcanism inferred from DSDP sites and seismic reflection profiling. Thus although other undated oceanic islands and seamounts in the Pacific, not crossed by the cruises, may possibly be of similar age to this event we have not plotted them in Fig. 5.

Discussion

We have documented a major geological event involving an extensive outpouring of ridge crest volcanism on the Pacific plate during the interval 90–120 Myr BP (Barremian to Turonian). This event was probably accompanied by deep-water volcanism. The area of the plate affected by the volcanism is $\sim 10^7$ km^2 (Fig. 5), which is significantly larger than areas of comparable volcanism on the continents (for example, western US since the Miocene[39]).

The origin of the ridge crest volcanism on the Pacific plate during the interval 90–120 Myr BP is not clear at present. The fixed or moving hot-spot hypothesis[40–42] cannot simply explain the volcanism unless a large number of contemporaneous hot spots are proposed along or near the former Pacific/Farallon and Pacific/Phoenix plate boundaries. The lithospheric fracture hypothesis[43] explains the widespread similarity in ages but cannot explain the volcanism unless a stress system is invoked which causes tensional failure of the plate in a direction generally parallel to the trend of the former Pacific/Farallon and Pacific/Phoenix plate boundaries. Thermal contraction of the oceanic plate[44], for example, would cause tensional failure which propagates in the direction of sea floor spreading.

An additional source of stress in the plates, however, are those associated with the driving mechanism of plate tectonics[45]. There is evidence, for example, from magnetic lineation patterns[36] that the 90–120 Myr BP volcanic event on the Pacific plate (Fig. 5) occurred at a time of relatively rapid spreading rate on the Pacific/Farallon ridge crest. This spreading rate increase, if it occurred[46], may have been accompanied by either the initiation of subduction or an increase in the convergence rate on another Pacific plate boundary. If this increase in convergence rate was accompanied by an increase in the driving force on the subducting plate[47], additional stresses may have been transmitted to the horizontal part of the plate. These stresses may have been sufficient to cause the Pacific plate to fracture at its weakest point and allow magma to flow from the underlying asthenosphere to the area around the ridge crest.

We have also documented the distribution of off-ridge volcanism on the Pacific plate (Fig. 4*b*). As some of the estimates of off-ridge volcanism are from the oldest part of the Pacific plate (Fig. 4*b*) this volcanism is therefore of late Jurassic or younger age. The main problem, however, is that with the exception of

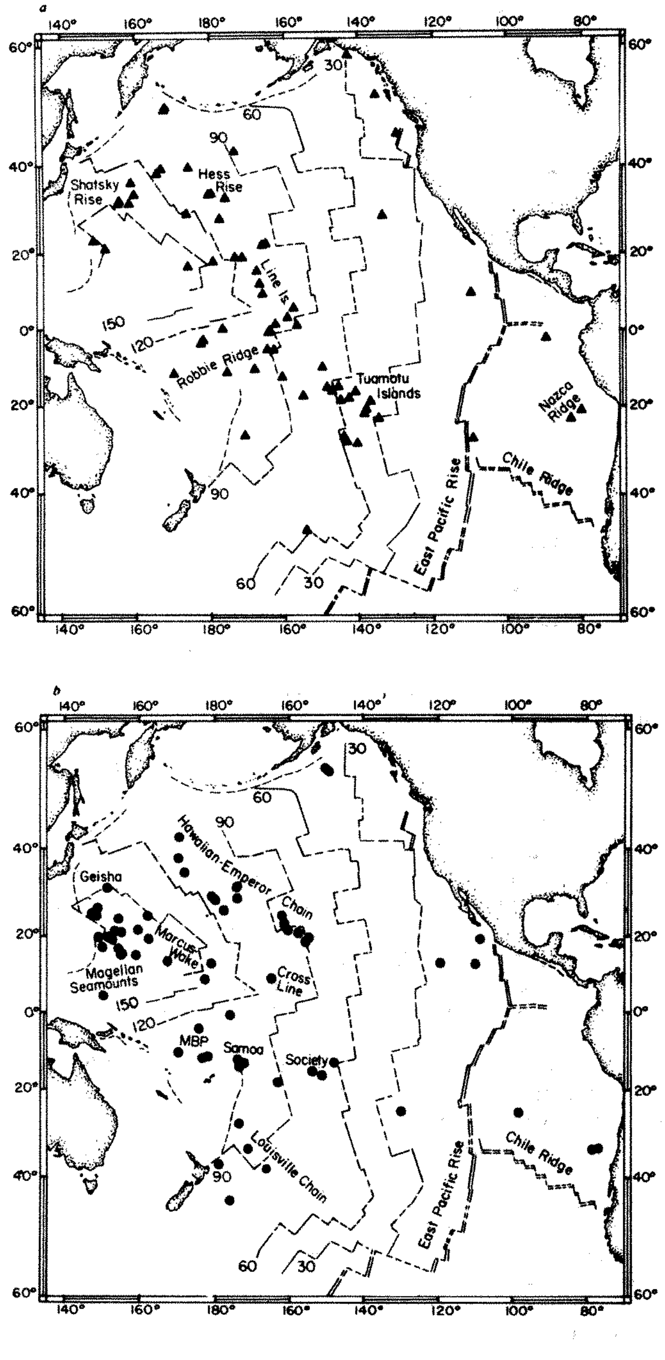

Fig. 4 Plot of estimates of ridge crest (*a*) and off-ridge volcanism (*b*) on the Pacific plate. The solid lines indicate isochrons based on observed magnetic lineations and DSDP sites and the dashed lines indicate interpolated isochrons based on tectonic reconstructions for the Pacific plate[22]. MBP, Melanesian border plateau[23].

the Hawaiian–Emperor seamounts and some features in the Geisha Guyots, where there is adequate geological control, the age of the off-ridge volcanism is generally unknown (for example, Table 1).

There is evidence, however, that at least part of the off-ridge volcanism in Fig. 4*b* may be of younger age than 90–120 Myr BP. The Line Islands ridge between latitudes 5°N and 15°N are intersected by the WNW–ESE Cross-Line Island trend which, based on bathymetry[4] and the estimated age of the off-ridge estimate at latitude 12°N longitude 165°W (Fig. 4*b*), are younger than the Line Islands ridge. The Melanesian border plateau, Samoa and Society Islands, which seem to form part of a broad WNW–ESE trend in off-ridge volcanism in the central Pacific (Fig. 4*b*), are probably younger than middle Cretaceous (see Table 1). The age of the off-ridge volcanism of the Magellan seamounts and Marcus–Wake Guyots is not known, however, although they are associated with similar WNW–ESE trends in bathymetry as the Cross-Line Island trend[4,24].

The presence of WNW–ESE trends in the bathymetry of features associated with off-ridge volcanism (Marcus–Wake Guyots, Magellan seamounts, Cross-Line Island trend) is of particular interest as they cross isochrons and are generally parallel to the direction of present day absolute Pacific plate motions. One possibility therefore is that the off-ridge volcanism is a surface expression of small-scale convection[48] in the underlying mantle. The ages of these features are not well enough known, however, and more studies need to be carried out before this possibility can be examined.

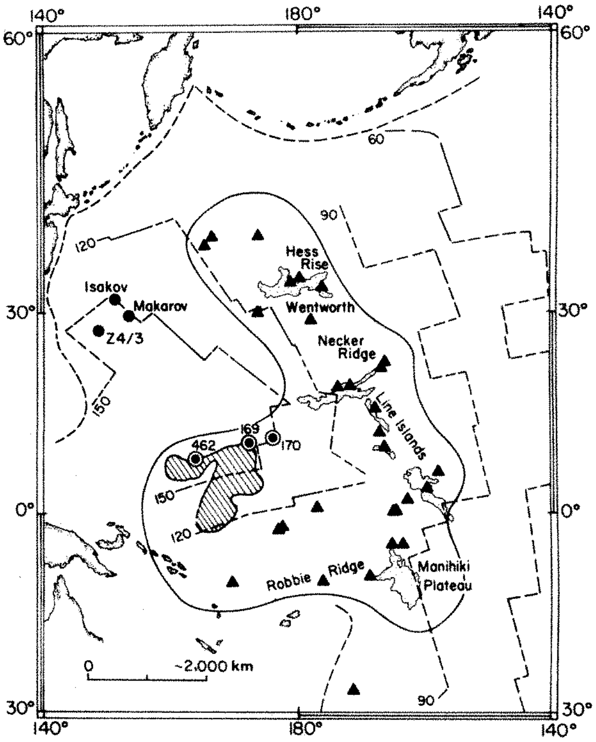

Fig. 5 Map summarising the inferred distribution of volcanism on the Pacific plate during the interval 90–120 Myr BP (Barremian to Turonian). ▲, Bathymetric features formed at a ridge crest; ●, features formed off-ridge. The ridge crest features probably originated on or near the Pacific/Farallon and Pacific/Phoenix ridge crests[36] and the off-ridge features originated in the interior of the Pacific plate away from the accreting plate boundaries. ◉, The deep water DSDP sites which were drilled on oceanic crust of probable late Jurassic age and sampled basaltic igneous rocks of Albian to Aptian age (100–110 Myr BP). The shaded area is the >600-m isopach of the seismic reverberant layer[38] in the region of DSDP sites 462 and 169 and the stippled area indicates the extent of shallow (<4,000 m)[24] sea floor in the region of the Manihiki plateau, Line Islands ridge, Necker ridge, Hess rise and Mid-Pacific mountains.

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In vitro and *in vivo* products of *E. coli* lactose permease gene are identical

Ruth Ehring & Konrad Beyreuther

Institut für Genetik der Universität zu Köln, Weyertal 121, D5000 Köln 41, FRG

J. Keith Wright & Peter Overath

Max-Planck-Institut für Biologie, Corrensstr. 38, D7400 Tübingen, FRG

The lacY *gene product synthesised* in vitro *is identical to lactose permease isolated from cytoplasmic membranes as determined by apparent molecular weight and N-terminal amino acid sequence. The amino acid composition of the* in vivo *product agrees well with that predicted from the DNA sequence. The data assign the translational start on the DNA sequence and demonstrate that this protein is processed only by deformylation but not by proteolytic cleavage at the N-terminus.*

In both bacterial and eukaryotic cells, most secreted proteins are initially synthesised as precursors with hydrophobic amino-terminal extensions which are removed by proteolytic cleavage during or after translocation of the protein through the membrane[1,2]. Such precursors have been demonstrated for some integral membrane proteins, such as the coat protein of the bacteriophage M13 (refs 3, 4), whereas in other cases the primary translation product formed *in vitro* seems to be identical to the membrane-associated *in vivo* product[5,6]. We describe here the *in vitro* synthesis of lactose permease (lactose carrier, product of the *Y* gene of the *lac* operon) of *Escherichia coli*[7] and show that the *in vitro* product is identical to permease isolated from the cytoplasmic membrane of this organism. As a model for the biosynthesis of a hydrophobic protein, lactose permease seems particularly attractive because this protein must attain a highly organised tertiary structure in the membrane in order to function as a lactose/proton-symporter[8].

Cell-free protein synthesis directed by hybrid plasmid pGM21

pGM21 is a hybrid plasmid between the vector pACYC 184 (ref. 9) and a previously described *lac Y* gene-containing fragment[10,11] integrated into the single site for the restriction enzyme *Eco*RI located in the gene conferring resistance to chloramphenicol. The fragment comprises about 2,300 base pairs and carries an intact *lac* promoter and operator region, an intact *Y* gene, but only minor parts of the *A* (thiogalactoside transacetylase), *Z*(β-galactosidase) and *I*(repressor) genes [genotype $lac\Delta(I)P^{+}O^{+}\Delta(Z)Y^{+}\Delta(A)$]. The restriction maps of this fragment and the *lac Y* gene-containing fragment sequenced in the preceding paper[12] are identical in the region of the *Y* gene. *In vivo*, pGM21-directed synthesis of lactose permease is inducible. The protein comprises up to 15% of the cytoplasmic membrane protein and is functionally intact as judged by transport and substrate binding experiments[10,13,14].

When added to a coupled transcription–translation system according to Zubay *et al.*[15], plasmid pGM21 efficiently promotes cell-free protein synthesis. In an incubation mixture containing ^{14}C-phenylalanine as the only radioactive amino acid, 11% of the label is found in a high molecular weight (MW) form, whereas in the absence of pGM21 only 0.1% of the input radioactivity becomes acid precipitable. An aliquot of the total incubation mixture was analysed on a polyacrylamide gel (Fig. 1*a*). It is clear that pGM21 directs the synthesis of one major product (slice nos 36–42, 33% of radioactivity) of apparent MW 30,000 ± 1,000. This radioactive peak corresponds in both mobility and width to purified lactose permease (prominently stained band in gel at the top of Fig. 1) or permease labelled with ^{3}H-*N*-ethylmaleimide in intact cytoplasmic membranes (Fig. 1*b*). Thus, the main product of the *in vitro* synthesis is identical to lactose permease within the resolution of the method.

As pGM21 contains an intact promoter–operator region, *in vitro* expression of the *Y* gene should be stimulated by cyclic AMP and reduced by *lac* repressor. Furthermore, repression should be relieved by addition of the inducer isopropyl β-D-thiogalactopyranoside (IPTG). Analysis of *in vitro* protein synthesis reaction mixtures on acrylamide gels demonstrates that the major product (apparent MW 30,000) is indeed subject to regulation by cyclic AMP and repressor (compare lanes 6–9 of Fig. 2). In terms of total incorporation of radioactive amino acids into protein (see Fig. 2 legend), cyclic AMP stimulates about twofold, repressor suppresses this synthesis by 60%, and addition of IPTG relieves the inhibition almost completely. These effects of cyclic AMP and repressor are small compared with control studies on *in vitro* synthesis of β-galactosidase directed by DNA from *lac* transducing phage λ in the same conditions. As quantitated by catalytic activity, the synthesis of this enzyme

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was reduced to less than 5% of the control by the omission of cyclic AMP or by addition of repressor (data not shown). The different behaviour of the two DNA preparations may be related to the use of circular DNA in the case of pGM21.

Depending on the conditions of gel electrophoresis, variable amounts of radioactivity in polypeptide material are distributed almost homogeneously throughout the high-MW region of the gel. As lactose permease has a marked tendency to aggregate, this material could at least in part represent *Y* gene product. This assumption is supported by the observation that the diffusely distributed radioactivity is also subject to regulation by cyclic AMP and repressor (Fig. 2). Moreover, co-electrophoresis of ^{3}H-labelled protein synthesised in the presence of repressor and IPTG and ^{14}C-labelled protein made in the presence of repressor only shows that the normalised radioactivity profiles essentially coincide from the start to the front of the gel (data not shown). Thus, repressor affects not only the synthesis of the main product (apparent MW 30,000) but also that of material of lower mobility and small polypeptides moving to the front. The diffusely distributed radioactivity in the high-MW region together with the permease band form about 60% of the total radioactivity on the gel.

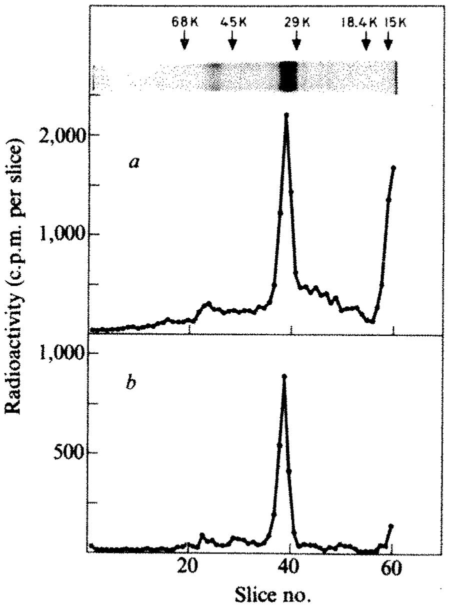

Fig. 1 Comparison of *in vivo* and *in vitro lac Y* gene products. *a*, Polyacrylamide-gel electrophoresis of *in vitro* products. A cell-free extract of *E. coli* K12 strain 514 (carrying a deletion of the *lac* operon) was used in a coupled transcription translation system as described previously[15,19]. First, the extract was incubated for 60 min at 0°C with 1 mM phenylmethanesulphonyl fluoride. To minimise accumulation of degradation products and of incomplete polypeptides, radiolabelling by ^{14}C-phenylalanine was limited to the interval from 15 to 25 min after the start of the reaction and was followed by a 5-min chase with a 25-fold molar excess of unlabelled phenylalanine. The 20 unlabelled amino acids were present at 0.22 mM each, pGM21 DNA at 30 μg ml^{-1} and polyethylene glycol 6000 at 25 mg ml^{-1} (all final concentrations). After the addition of ^{14}C-phenylalanine to 100 μCi ml^{-1}, the concentration of this amino acid was 0.36 mM. A further incubation for 10 min at 37°C with DNase I and RNase A (each added to a final concentration of 25 μg ml^{-1}) terminated the reaction. The complete reaction mixture incorporated 10.5% of the ^{14}C-phenylalanine into material insoluble in hot trichloroacetic acid. In the absence of pGM21 DNA, only 0.1% of the label was incorporated. An aliquot of 3 μl synthesis mixture was mixed with 27 μl sample buffer, incubated for 30 min at room temperature and applied to a slab gel (compare with ref. 20, 14% acrylamide–0.12% bisacrylamide). Free radioactive phenylalanine was removed during staining with Coomassie brilliant blue R 250. The figure shows the radioactivity distribution in sections of the gel counted as described previously[13]. *b*, Radioactivity distribution of a cytoplasmic membrane preparation in which permease was labelled with ^{3}H-*N*-ethylmaleimide[13,21] The top of the figure shows electrophoresis of lactose permease isolated in an inactive state from strain T206 (ref. 16). Arrows refer to marker proteins (bovine serum albumin, MW 68,000; ovalbumin, 45,000; carbonic anhydrase, 29,000; β-lactalbumin, 18,400; haemoglobin, 15,000.

Table 1 Amino acid composition of *lac* permease

Amino acid	No. of residues	
Asp	20.96	(6)
Asn		(16)
Thr	19.10	(19)
Ser	27.20	(29)
Glu	21.79	(11)
Gln		(11)
Pro	11.65	(12)
Gly	35.28	(36)
Ala	34.35	(35)
Cys	6.55	(8)
Val	28.70	(29)
Met	13.50	(14)
Ile	28.67	(33)
Leu	49.84	(54)
Tyr	11.67	(14)
Phe	35.40	(56)
His	4.28	(4)
Lys	12.05	(12)
Arg	12.56	(12)
Trp	4.72	(6)
Sum		(417)

Amino acids were analysed as described elsewhere[22] on a Beckman 121M amino acid analyser. The numbers given for valine, isoleucine, leucine and phenylalanine were derived from 192-h hydrolysates. Cysteine was analysed as cysteic acid. The integral values in parentheses are based on the DNA sequence[12].

A control experiment is shown in Fig. 2 (lanes 3, 4). The vector pACYC184 codes for one major product concentrated in a sharp band of MW 25,000±2,000. We attribute this band to the expression of the chloramphenicol resistance gene[9] which is inactivated by insertion of the *lacY*-containing fragment. Therefore, this product is lacking in pGM21-directed *in vitro* synthesis.

Amino-terminal sequence of the *lacY* gene product synthesised *in vitro*

The amino acid sequence of the product encoded by pGM21 DNA *in vitro* was partially elucidated. The material synthesised in the presence of ^{14}C-phenylalanine, ^{3}H-lysine, ^{14}C-leucine or ^{35}S-methionine was subjected to automated sequence analysis (Fig. 3). These four amino acids appear in the following positions—Phe: 9, 12, 15, 16, 17, 18, 20, 21, 27, 29 and 30; Lys: 5 and 42; Leu: 4, 14 and 34; Met: 1, 11 and 23. These assignments agree completely with the prediction of the DNA sequence[12] and thus identify the major protein synthesised from pGM21 DNA *in vitro* as the product of the *lacY* gene. This species represents the predominant product of cell-free synthesis, as sequence analysis does not reveal the presence of amino acids in positions other than those predicted by the DNA sequence.

Neither the permease nor another protein component exists predominantly with a formylated N-terminus. The ^{35}S-methionine liberated after the first degradation cycle is 74% of that observed when the product is chemically deformylated (Fig. 3*d*). The newly deblocked material contains no additional methionine residues up to position 21, suggesting that no different sequences are made available for analysis. Thus, methionine-1 is probably encoded by the initiation codon of the *Y* gene, and the protein is efficiently deformylated in the cell-free extract. The yield of radioactive methionine released from the deformylated material in the first sequencing step was 72% of that expected from the methionine content of permease (see Table 1 and Fig. 3). The yields of the more stable amino acids Phe, Leu and Lys were 82–91% (Fig. 3).

The *lac* permease synthesised *in vitro* was biosynthetically labelled with 15 amino acids and submitted to direct microsequence analysis (Fig. 4). After 42 sequencing steps, 34 residues could be identified. The unassigned positions correspond to

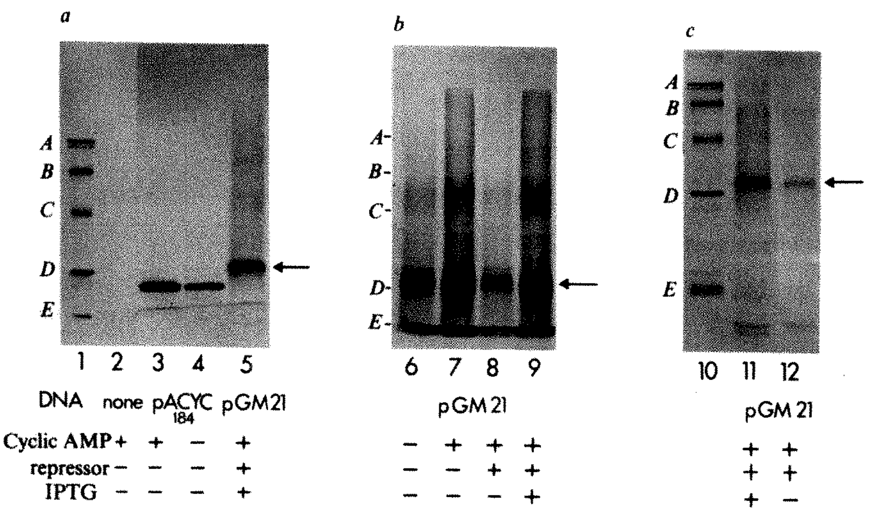

Fig. 2 Regulation of expression of pGM21 *in vitro*. DNA-directed cell-free protein synthesis was carried out as described for Fig. 1 with the following alterations. Radiolabelling for samples of *a* and *b* was continuous throughout a 30-min synthesis period with a mixture of ^{14}C-labelled amino acids (algal protein hydrolysate, Amersham-Buchler) added to the complement of 20 nonradioactive amino acids all at 0.2 mM final concentration. Additions indicated below the figure were as follows: pACYC184 (vector) or pGM21 (plasmid carrying *lacY*) DNA (30 μg ml^{-1}), cyclic AMP (1 mM), *lac* repressor purified to the final DEAE-cellulose step (ref. 22, 8.5 μg ml^{-1} for samples in *b* and 30 μg ml^{-1} for samples in *c*), and IPTG (0.2 mM). Incorporation of radioactivity into hot trichloroacetic acid-insoluble material was 1, 13, 5 and 18% for lanes 2–5; 11, 24, 9 and 22% for lanes 6–9 and 10 and 3% for lanes 11 and 12, respectively. Aliquots of 5 μl (*a* and *b*) or 2.5 μl (*c*) were applied to 12% (*a* and *b*) or 20% (*c*) polyacrylamide gels without (*b*) or with (*a* and *c*) prior heating for 5 min to 95°C. ^{14}C-methylated marker proteins (*A–E* lanes 1 and 10): phosphorylase (MW 92,500, bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000) and cytochrome *c* (13,000).

the amino acids asparagine, histidine, tryptophan and serine, according to the DNA sequence. With the exception of serine, these amino acids were not present in the mixture of radioactive amino acids used for the labelling *in vitro*. The partial sequence obtained is: H_2N-Met-Tyr-Tyr-Leu-Lys-X-Thr-X-Phe-X-Met-Phe-Gly-Leu-Phe-Phe-Phe-Phe-Tyr-Phe-Phe-Ile-Met-Gly-Ala-Tyr-Phe-Pro-Phe-Phe-Pro-Ile-X-Leu-X-Asp-Ile-X-X-Ile-X-Lys (42 residues; X, not determined). These residues correspond exactly to those predicted by the DNA sequence.

The ability to determine a unique sequence directly from the reaction mixture underscores the homogeneity of the *in vitro* product and the usefulness of pACYC184 as a vector provided only one gene is expressed from an insert into the *Eco*RI site.

Comparison of *in vivo* and *in vitro* products

lac permease isolated from the cytoplasmic membrane of the plasmid-containing *E. coli* strain T206 in an inactive state (see gel on top of Fig. 1 (ref. 16)) was degraded manually without prior chemical deformylation. Residues 1–9 and 11–15 of the *in*

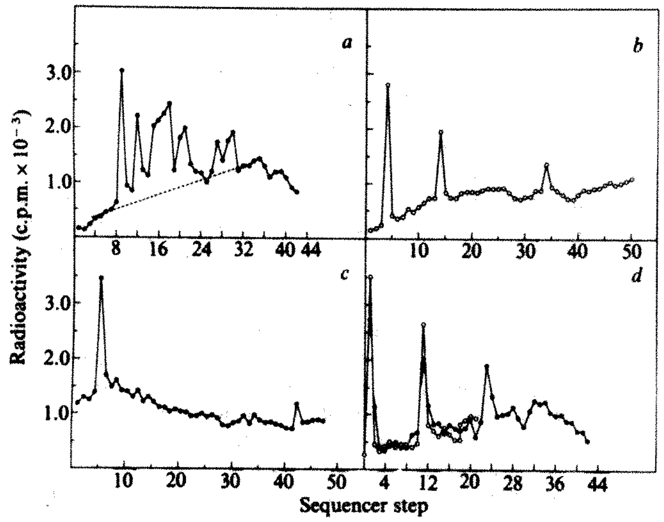

Fig. 3 Amino acid sequence analysis of the total cell-free products programmed by pGM21 plasmid DNA. The products were labelled with one radioactive amino acid at a time: *a*, ^{14}C-phenylalanine (256,000 c.p.m. in the sample analysed); *b*, ^{14}C-leucine (231,000 c.p.m.); *c*, ^{3}H-lysine (62,000 c.p.m.); *d*, ^{35}S-methionine (110,000 c.p.m. in both experiments). Total synthesis mixtures were dialysed against 50 mM ammonium bicarbonate, *p*H 8.5, lyophilised, chemically deformylated[23] (with the exception of *d*, ●), dialysed against 10% formic acid in the presence of 4 mg oxidised chicken lysozyme and subjected to automated Edman degradation in an updated Beckman model 890B sequencer equipped with a Sequemat P-6 automatic converter. Degradations were carried out in the presence of precycled polybrene (1.5 mg) using a 0.2 M quadrol program with double cleavage[24]. The radioactivity in the fraction obtained at each cycle of degradation was determined in 10 ml Aquasol-2 (NEN) containing 2% methanol with a Packard Tri-Carb liquid scintillation spectrometer. The analysis of the phenyl thiohydantoin derivatives of the carrier lysozyme was as described elsewhere[22,25]. Repetitive yields of the protein carrier and of the radioactively labelled materials ranged between 94 and 96%. Absolute yields corrected for background and carry over[26] of the radioactive amino acids studied were 90.6% (*a*), 84.7% (*b*), 81.6% (*c*) and 72.1% (*d*, ○) for chemically deformylated products and 53.0% without chemical deformylation (*d*, ●).

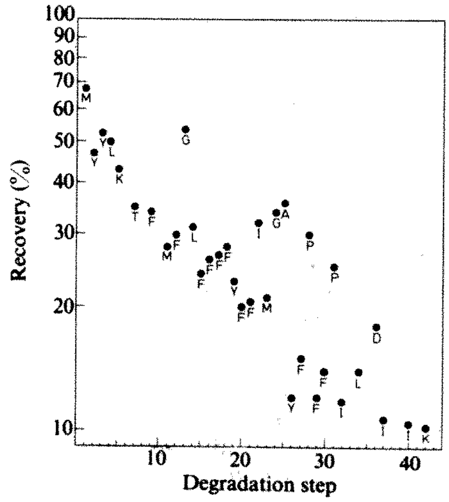

Fig. 4 Partial amino-terminal sequence (residues 1–42) of *lac* permease synthesised *in vitro*. Cell-free products programmed by pGM21 plasmid DNA were labelled with ^{35}S-methionine and the following ^{14}C-labelled amino acids: aspartic acid (D), threonine (T), serine (S), glutamic acid (E), proline (P), glycine (G), alanine (A), valine (V), isoleucine (I), leucine (L), tyrosine (Y), phenylalanine (F), lysine (K) and arginine (R) as described for Fig. 2. The sedimentable material of the protein synthesis mixture obtained by centrifugation at 100,000*g* for 60 min[27] was treated and sequenced as described for Fig. 3. The radioactive phenylthiohydantoin derivatives at each sequencer cycle were identified by radiolabelled amino acid analysis after back-hydrolysis with 6M HCl containing 0.2% $SnCl_2$ (ref. 28) using the Beckman 121M amino acid analyser with effluent collection in principle as described by McKean *et al.*[29]. The efficiency of radioactive counting was 33% for regenerated ^{14}C-labelled amino acids. The recoveries based on specific activity measurements are plotted on a logarithmic scale against step number[26]. The relative specific activities of the amino acids were estimated by radiolabelled amino acid analysis of particulate matter after acid hydrolysis and are based on the amino acid composition of *lac* permease deduced from the DNA sequence[12]. No correction for hydrolytic loss of the regenerated amino acids was made. Total radioactivity loaded on the sequencer was 2 × 10^{6} c.p.m.

	1				5					10					15
a Protein in vitro	Met	Tyr	Tyr	Leu	Lys	X	Thr	X	Phe	X	Met	Phe	Gly	Leu	Phe
b Protein in vivo	Met	Tyr	Tyr	Leu	Lys	Asn	Thr	Asn	Phe	X	Met	Phe	Gly	Leu	Phe
c DNA sequence	Met	Tyr	Tyr	Leu	Lys	Asn	Thr	Asn	Phe	Trp	Met	Phe	Gly	Leu	Phe

Fig. 5 Summary of sequence data on *lac* permease. *a*, Partial amino-terminal sequence analysis of the *in vitro* synthesised permease (see Figs 3, 4); *b*, automated (underlined residues) and manual sequence analysis of permease isolated from cytoplasmic membranes; *c*, from DNA sequence (12). *X*, not determined.

vivo product could be determined by the 4-*N,N*-dimethylaminoazobenzene 4′-isothiocyanate/phenylisothiocyanate double-coupling method of Chang *et al.*[17]. Automated Edman degradation was carried out for five cycles to determine the amount of the amino-terminal residue which could be released by sequential degradation. Methionine was found in the first sequencing step with a yield of 65%. Figure 5 compares the amino-terminal sequences of permease synthesised *in vitro* and *in vivo* with that deduced from the DNA sequence of the *Y* gene. The agreement of the N-terminal sequences confirms the identity of these two products. The amino acid composition determined for the *in vivo* product is very similar to that deduced from the DNA sequence (Table 1). There is good agreement of the values for Glx, Pro, Gly, Val and Arg, each of which occur twice in the C-terminal 15 residues of the permease as deduced from the DNA sequence[12]. Any C-terminal processing would then seem unlikely also. The value for phenylalanine is, however, too low. Even after 192 h of hydrolysis, only 35 of the expected 52 phenylalanine residues are found. The unusually high number of potentially acid-resistant Phe-Phe linkages in the sequence[12] may account for the low recovery of phenylalanine from the acid hydrolysate[18] of the *lac* permease.

Conclusions

The N-terminal sequence of both the *in vitro* and *in vivo* products of the *lac Y* gene unambiguously assigns the translational start of the protein to the methionine codon corresponding to base pairs 106–108 in the sequence of Büchel *et al.*[12] rather than to the alternative codons at base pairs 136–138 or 172–174. The identity of the *in vivo* and *in vitro* synthesised proteins in terms of apparent MW and N-terminal sequence shows that this protein is not processed by proteolytic cleavage at the N-terminus. Moreover, both products are deformylated at the N-terminal methionine, suggesting that the amino-terminal residue is not directly inserted into the membrane and thus shielded against the action of the deformylating enzyme. Other membrane-associated *in vivo* products of bacterial and mitochondrial origin have been shown to retain the fMet residue[6]. There is good agreement between the amino acid composition of the *in vivo* product and that predicted from the DNA sequence. Furthermore, preliminary experiments show that cleavage of the *in vivo* product by cyanogen bromide yields the expected number of polypeptide fragments. These observations make it unlikely that processing at the C-terminus occurs. The MW predicted from the DNA sequence (46,500 (ref. 12)) is much larger than the apparent MW of permease on acrylamide gels. This behaviour could be attributed to increased binding of SDS, as expected from the high hydrophobicity of this protein.

As the cell-free extract used in our studies has not been freed of membranes, it is not clear whether the protein becomes associated with membranes during or after synthesis. Preliminary experiments indicate that the *in vitro* product is readily pelleted by centrifugation but remains more susceptible to proteolysis by trypsin than the functional protein in the membrane, suggesting that the major part of the *in vitro* formed protein is in a non-membranous environment. Whether the lactose permease is synthesised *in vivo* on membrane-bound ribosomes is particularly relevant in view of the location of the *Y* gene between two genes which are probably translated by free ribosomes. Alternatively, the intitial formation of a soluble (possibly modified) form of the protein which spontaneously inserts into the membrane cannot be excluded. Obviously , the highly hydrophobic N-terminus of the permease may serve as an initial recognition site for integration into the membrane.

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Sequence of the lactose permease gene

Dagmar E. Büchel, Bruno Gronenborn & Benno Müller-Hill
Institute for Genetics, University of Cologne, Weyertal 121, D-5000 Cologne 41, FRG

The nucleotide sequence of the lacY *gene coding for lactose permease (M protein) in* Escherichia coli *has been determined. The sequence includes the intergenic regions between the* lacZ *(β-galactosidase) and* lacY *genes as well as the region between the* lacY *and* lacA *(transacetylase) genes. Lactose permease is predicted to consist of 417 residues (71% nonpolar), resulting in a protein with a molecular weight of 46,504. The reading frame was confirmed by the sequence of a nonsense mutation changing codon 33 from UGG to UAG.*

LACTOSE PERMEASE is a protein which transports and accumulates galactosides through the inner membrane into *Escherichia coli*. It was discovered by Rickenberg *et al.*[1], who described it as the product of a structural gene *Y* of the *lac* system of *E. coli* capable of transport and intracellular accumulation of exogenous galactosides. The product of the *lacY* gene (M protein) was later identified and partially purified in inactive form by Fox and Kennedy[2]. Recently, a plasmid has been described which overproduces *lac* permease[3]. However, the isolation of inactive *lac* permease has been difficult and the isolation of active permease has not been achieved.

lac permease probably operates without the help of any other protein. We considered that an understanding of its structure would lead to the understanding of its function, transport, therefore we have determined the nucleotide sequence of the *lacY* gene. The sequence allowed the prediction of the primary structure of *lac* permease: the primary translation product consists of 417 residues. Ehring *et al.* (see accompanying article[4]) have shown by protein sequence analysis that *lac* permease synthesised *in vivo* and *in vitro* starts with the native sequence predicted by us.

Cloning of the *lacY* gene

We used the transducing phage *λh80dlacI857Ssus7* (ref.5), carrying the wild-type *lac* operon, as the source of *lacY* DNA. A DNA fragment containing the *lacY* gene was obtained by restriction endonuclease digestion with *Eco*RI and cloned in the filamentous single-stranded DNA phage *M13*mp2 (refs 6, 7) (for details see Fig. 1 legend). We expected the insert to be 18,000 base pairs long. The DNA length of the cloning vehicle *M13*mp2 is about 7,200 base pairs, and so an insert of 18,000 base pairs would increase its length about 3.5-fold. Phages of that size would replicate rather slowly, favouring the accumulation of phages carrying deletions in the population. Indeed, we found inserts to have a size of about 5,500 base pairs. One of them was again reduced *in vivo* to 2,100 base pairs (*M13*mp2561). This insert contained the last 17 codons of the *lacZ* gene, the *lacY* gene and part of the *lacA* gene. We also cloned it in the inverted orientation in *M13*mp2 and in the mini ColE1 plasmid pVH51 (ref. 8). The latter plasmid (pGB2601) served as a source of smaller restriction subfragments which were required for sequence analysis.

Expression of the *lacY* gene under control of the *lac* promotor of the phage *M13*mp2 was shown on minimal agar plates containing 0.2% melibiose (see Fig. 1*a* legend). Although we had expected bacteria carrying about 200 copies of the replicative form (RF) of *M13*mp2561 DNA to overproduce *lac* permease, in such bacteria, we found that the transport activity was only 50% of that measured in a strain carrying one copy of the *Y* gene on a F′-*lac pro* episome. Therefore, it was possible that we had selected one or several mutations which reduced either the amount or the activity of permease. It was thus essential to compare the DNA sequence of the *lacY* gene cloned in conditions preventing expression with the sequence of the *Y* gene cloned under selective pressure for expression. We inserted a 3,500-base pair *Eco*RI-*Bgl*II fragment of *λh80dlacI857Ssus7* in *M13*mp2 DNA (Fig. 1*b*). This fragment carried the *lacY* and the *lacA* genes. The tranducing phages thus obtained (*M13*mp2702) were grown in a cap^-cya^- strain which reduces *lac* expression to about 2% (ref. 9). The inverted orientation of the fragment was derived by inserting it into *M13*mp3 DNA[7] (*M13*mp3110).

To confirm the reading frame of the *lacY* gene and to define the start of permease, the Y^- amber mutation NE4, mapping in the fourth deletion group of the *Y* gene[10], was directly crossed into *M13*mp2561 from an F′-*lac pro* episome (see Fig. 1 legend).

DNA sequence analysis

We determined the nucleotide sequence of the *lacY* gene using the methods described by Maxam and Gilbert[11] and by Sanger *et al.*[12]. For the chemical modification technique[11] we prepared restriction subfragments of the 2,100-base pair insert of pGB2601. We constructed a restriction map of *Msp*I, *Hha*I, *Hae*III and *Mbo*II restriction endonuclease cuts (Fig. 2). Other restriction cuts were deduced during sequence analysis. Single-stranded DNA of *M13*mp2561, mp2601, mp2702 and mp3110 was prepared[13] from the respective phages and used as template for sequence analysis according to the method of Sanger *et al.*[12] modified as described by Schreier and Cortese[13]. Primers were prepared as restriction subfragments from the 2,100-base pair insert of pGB2601. Details of the sequencing procedures are given in Fig. 2 legend.

The *lacY* gene sequence of the 2,100-base pair insert, including the intergenic region between *lacY* and *lacA*, was determined on both complementary strands using primarily the enzymatic sequencing method[12]. The sequence of the end of the *lacZ* gene and the intergenic region between *lacZ* and *lacY* was obtained from one strand by both the chemical[11] and the enzymatic[12] procedures, and the beginning of the *lacA* gene from one strand was obtained by the technique of Sanger *et al.*[12]. The *Y* gene sequence of the 3,500-base pair fragment and that of the amber mutant NE4 were determined from one strand by the method of Sanger *et al.*[12]. A comprehensive description of the sequencing experiments is given in Fig. 2 legend.

Nucleotide sequence

The DNA sequence (Fig. 3) beginning with the *Eco*RI cut at the end of the *lacZ* gene shows the 17 C-terminal codons of *lacZ*. The sequence is compatible with the protein sequence determined by Fowler and Zabin[14]. The end of the *Z* gene is characterised by an unusually strong stop signal: three ochre codons in a row.

In the DNA following the *Z* gene only one frame is free of barriers. To confirm the reading frame of the *lacY* gene we sequenced DNA carrying the amber mutation NE4 mapping in a region of the gene specifying the N-terminus of permease[10]. Gels with the respective sequences of NE4 and wild-type DNA are shown in Fig. 4. The guanosine at position 203 is changed to an adenosine, changing a Trp codon (TGG) of the permease

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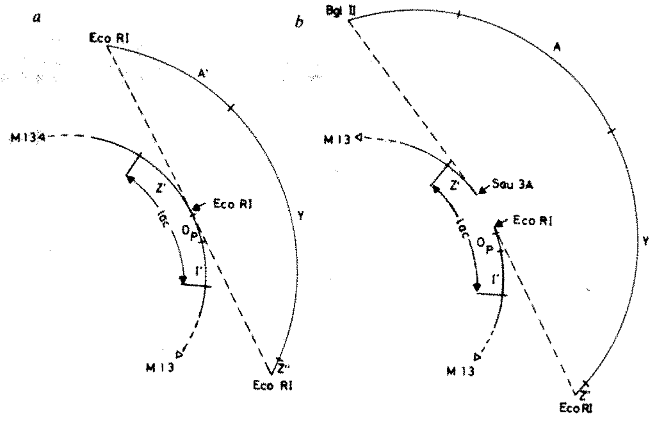
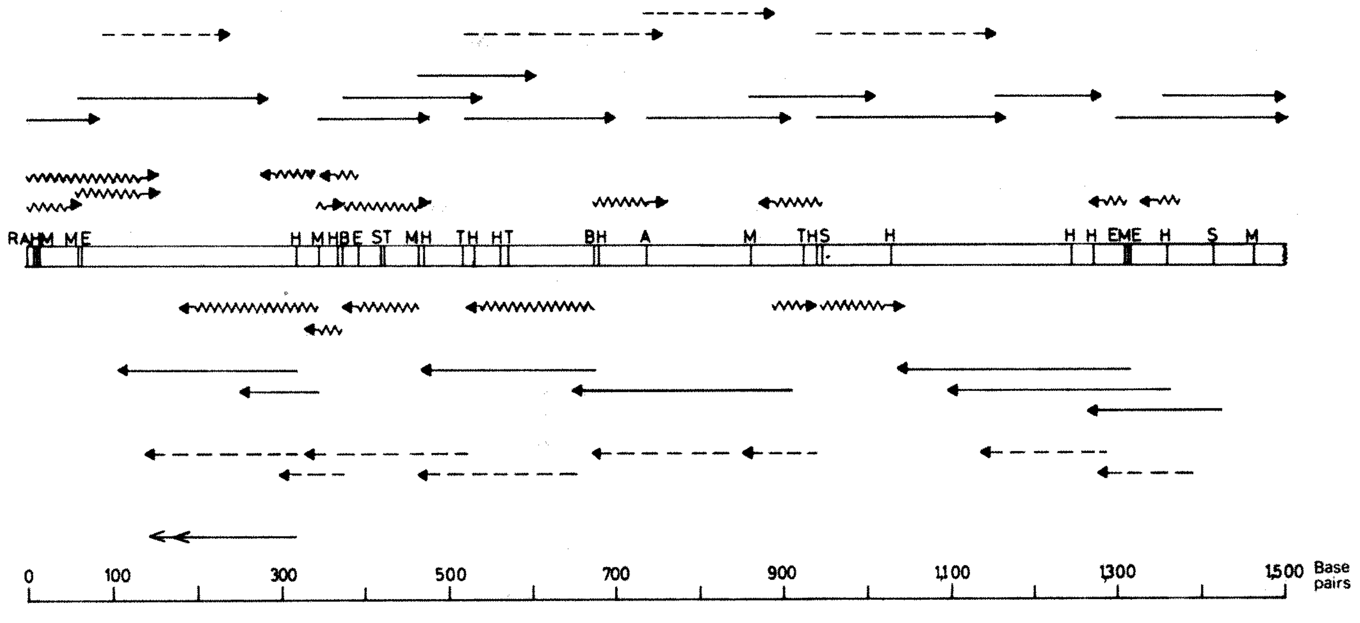


Fig. 1 Cloning of the *lacY* gene. *a*, *Eco*RI restriction endonuclease fragments of the *lac* transducing phage *λh80dlaccI857Ssus7* DNA were ligated with *M13*mp2 RF DNA linearised by *Eco*RI using T_4 DNA ligase according to Bolivar *et al.*[25] The ligation mixture was used to transform strain 75X [*lac, pro*]Δ *thi, strA, ϕ80dlac* [*Y, A, tonB, trp*]Δ 75X (ref. 10) as described by Mandel and Higa[26]. Bacteria were selected for growth at 42 °C in supplemented minimal medium[27] containing 1% melibiose as carbon source. Cells expressing lactose permease activity will grow in these conditions and enrich in the culture. Due to the F^- phenotype of strain, 75X *M13* phages can be extruded from the cells but do not re-infect uninfected bacteria. This prevents an excessive propagation of non-hybrid phage as well as the enrichment of the progeny originating from only a few transducing phage by re-infection. Phage from the supernatant of enriched cultures were used to infect strain MUA2 [*lac, pro*]Δ *thi nalA, F′ lac* $I^Q Y^-$ ΔMUA2 pro^+ (ref. 10) and plated on minimal melibiose plates to grow at 42 °C. Single colonies were assayed for the release of *lacY* transducing *M13* phages in the following way. The supernatants of 1-ml cultures were spotted onto a lawn of indicator strain 50–15 [*lac, pro*]Δ *thi, strA, F′ lac* I^Q Y^- ΔMUA2 pro^+ on minimal melibiose plates containing 0.02% streptomycin to select against the former host. The plates were incubated for about 18 h at 33 °C and then at 42 °C for an additional overnight growth period. In these conditions *M13*mp2 phage give rise to slightly turbid 'plaques' whereas *lacY* transducing phage *M13* cause accelerated growth of the infected cells, resulting in a very turbid plaque with a significant halo of bacterial growth at its borderline. In this kind of plaque test, *M13*mp2 *lacY* tranducing phage can easily be distinguished. In addition, suppression of *lacY* amber mutants and recombination to $lacY^+$ can be monitored directly by the plaque morphology as described below. Two *lacY* transducing *M13* phages from different enrichment cultures were obtained. The length of the inserted DNA was determined to be 5,500 base pairs according to its mobility on 1% agarose gels. As the insert, although already shortened by a large deletion, contained four times more DNA than the approximate length of the *lacY* gene, an additional deletion was selected *in vivo*. This final *lacY* transducing *M13*mp2 derivative contained an insert of 2,100 base pairs (*M13*mp2561). For the sequence analysis according to Sanger *et al.*[12], a *M13 lacY* derivative with the insert in the inverted orientation was constructed by cutting *M13*mp2561 DNA with *Eco*RI and then ligating. After transformation, cells were assayed for plaque-forming centres in the *lacY* transduction test described above. Phages carrying the *Y* gene insert in the inverted orientation gave rise to a mottled plaque morphology due to occasional recombination of the Y^- host to wild type during plaque development (*M13*mp2601). A derivative of *M13*mp2561 carrying the Y^- amber mutation NE4 (ref. 10) was derived by propagating the phage in a strain that carried the mutation on a F′-*lac pro* episome. *lacY*-negative *M13* transucing phages were identified in the plaque test described above. The suppressible character of the mutation was shown by its intermediate phenotype between Y^- and Y^+ using a *supF* host. The correct map position was confirmed by deletion mapping against a set of *lacY* deletions[10] and the failure to recombine with the original NE4 mutation. *b*, To obtain clones in non-expressing conditions a 3,500-base pair *Bgl*II–*Eco*RI fragment was prepared from plasmid pBR322-Y46, a derivative of pBR322 (ref. 25) containing a 12,000-base pair *Eco*RI–*Bam*HI insert of *λh80dlaccI857Ssus7* including the *lacY* gene. *M13*mp2 RF DNA was linearised with *Eco*RI, partially digested with *Sau*3A and ligated to the 3,500-base pair *Eco*RI-*Bgl*II fragment. The cap^- cya^- strain CA7914-3 [*lac, pro*]Δ cap^-cya^-*thi strA F′ lac* I^Q pro^+ was transformed. In induced conditions this strain allows only 2% expression of the *lac* operon[9], in repressed conditions in the I^Q (*lac* repressor overproducing) situation, even less. Hybrid phages were assayed by the plaque morphology test described above; *lacY* transducing *M13* phage showed the typical very turbid plaque (*M13*mp2702). To obtain a phage with the *Eco*RI–*Bgl*II insert an inverted orientation, the fragment was inserted into *M13*mp3 (ref. 7) in the same way as described above. *lacY*-transducing phage of this type give rise to mottled plaques in the described conditions (*M13*mp3110).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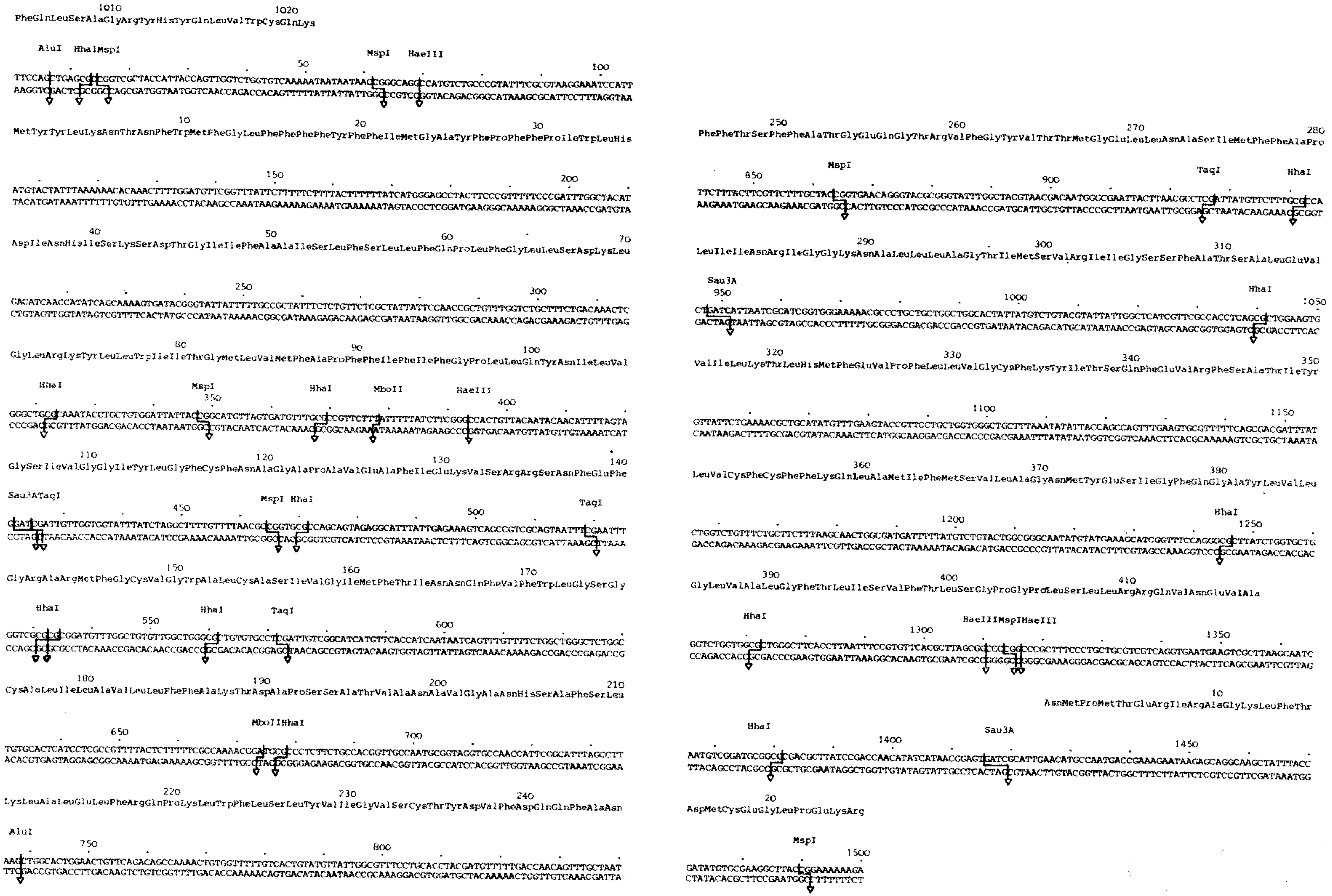
0
100
300
500
700
900
1100
1300
1500
Base pairs

Fig. 2 Restriction map of the *lacY* gene. The sequenced part of the 2,100- and the 3,500-base pair fragments is shown. A (*Alu*I), B (*Mbo*II), E (*Hae*III), H (*Hha*I), M (*Msp*I), R (*Eco*RI), S (*Sau*3A) and T (*Taq*I) denote the respective restriction cuts. For the Maxam–Gilbert sequencing technique[11] restriction fragments were labelled at the 5′-end with [γ^{32}–P]ATP (NEN) by polynucleotide kinase or at the 3′-end by filling in with the suitable [α-^{32}P]dNTP (NEN) using the DNA polymerase I Klenow fragment. For the sequencing method with chain-terminating inhibitors[12,13], templates were prepared according to Schreier and Cortese[13], restriction subfragments of the *lacY* insert of pGB2601 were used as primers. The same restriction fragments were hybridised to the templates carrying the inserts in opposite orientation so as to obtain the sequence of both complementary strands. Primers of more than 80 base pairs were reduced in length by treatment with exonuclease III before hybridisation with the template (R. Roberts quoted in ref. 13). This yields sequence information on about half of the length of the primer itself. The beginning, direction and extent of the sequence analysis of different parts are indicated by arrows. Arrows above or below the restriction map represent the sequences of the complementary strands determined (5′-end on left-hand side above the map). Wavy arrows represent sequences of the 2,100-base pair insert obtained according to Maxam and Gilbert, and straight arrows those obtained according to Sanger *et al.* Dashed arrows indicate sequences determined from *M13*mp2701 and *M13*mp3110 by the technique of Sanger *et al.*; the double arrow represents the sequence determined from the phage carrying the *lacY* amber mutation NE4 (*M13*mp2561-NE4) according to Sanger *et al.*


1010 1020
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AluI HhaIMspI MspI HaeIII
50 100
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AAGGTCGACTCGCGGCCAGCGATGGTAATGGTCAACCAGACCACAGTTTTTATTATTATTGGCCCGTCCGGTACAGACGGGCATAAAGCGCATTCCTTTAGGTAA
10 20 30
MetTyrTyrLeuLysAsnThrAsnPheTrpMetPheGlyLeuPhePhePhePheTyrPhePheIleMetGlyAlaTyrPheProPhePheProIleTrpLeuHis
150 200
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40 50 60 70
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250 300
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80 90 100
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HhaI MspI HhaI MboII HaeIII
350 400
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CCCGACGCGTTTATGGACGACACCTAATAATGGCCGTACAATCACTACAAACGCGGCAAGAAATAAAAATAGAAGCCCGGTGACAATGTTATGTTGTAAAATCAT
110 120 130 140
GlySerIleValGlyGlyIleTyrLeuGlyPheCysPheAsnAlaGlyAlaProAlaValGluAlaPheIleGluLysValSerArgArgSerAsnPheGluPhe
Sau3ATaqI MspI HhaI TaqI
450 500
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150 160 170
GlyArgAlaArgMetPheGlyCysValGlyTrpAlaLeuCysAlaSerIleValGlyIleMetPheThrIleAsnAsnGlnPheValPheTrpLeuGlySerGly
HhaI HhaI TaqI
550 600
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CCAGCGCGCGCCTACAAACCGACACAACCGACCCGCGACACACGGAGCTAACAGCCGTAGTACAAGTGGTAGTTATTAGTCAAACAAAAGACCGACCCGAGACCG
180 190 200 210
CysAlaLeuIleLeuAlaValLeuLeuPhePheAlaLysThrAspAlaProSerSerAlaThrValAlaAsnAlaValGlyAlaAsnHisSerAlaPheSerLeu
MboIIHhaI
650 700
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ACACGTGAGTAGGAGCGGCAAAATGAGAAAAAGCGGTTTTGCCTACGCGGGAGAAGACGGTGCCAACGGTTACGCCATCCACGGTTGGTAAGCCGTAAATCGGAA
220 230 240
LysLeuAlaLeuGluLeuPheArgGlnProLysLeuTrpPheLeuSerLeuTyrValIleGlyValSerCysThrTyrAspValPheAspGlnGlnPheAlaAsn
AluI
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250 260 270 280
PhePheThrSerPhePheAlaThrGlyGluGlnGlyThrArgValPheGlyTyrValThrThrMetGlyGluLeuLeuAsnAlaSerIleMetPhePheAlaPro
MspI TaqI HhaI
850 900
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LeuIleIleAsnArgIleGlyGlyLysAsnAlaLeuLeuLeuAlaGlyThrIleMetSerValArgIleIleGlySerSerPheAlaThrSerAlaLeuGluVal
Sau3A HhaI
950 1000 1050
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GACTAGTAATTAGCGTAGCCACCCTTTTTGCGGGACGACGACCGACCGTGATAATACAGACATGCATAATAACCGAGTAGCAAGCGGTGGAGTCGCGACCTTCAC
320 330 340 350
ValIleLeuLysThrLeuHisMetPheGluValProPheLeuLeuValGlyCysPheLysTyrIleThrSerGlnPheGluValArgPheSerAlaThrIleTyr
1100 1150
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CAATAAGACTTTTGCGACGTATACAAACTTCATGGCAAGGACGACCACCCGACGAAATTTATATAATGGTCGGTCAAACTTCACGCAAAAAGTCGCTGCTAAATA
360 370 380
LeuValCysPheCysPhePheLysGlnLeuAlaMetIlePheMetSerValLeuAlaGlyAsnMetTyrGluSerIleGlyPheGlnGlyAlaTyrLeuValLeu
HhaI
1200 1250
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390 400 410
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HhaI HaeIIIMspIHaeIII
1300 1350
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CCAGACCACCGCGACCCGAAGTGGAATTAAAGGCACAAGTGCGAATCGCCGGGGCCGGGCGAAAGGGACGACGCAGCAGTCCACTTACTTCAGCGAATTCGTTAG
10
AsnMetProMetThrGluArgIleArgAlaGlyLysLeuPheThr
HhaI Sau3A
1400 1450
AATGTCGGATGCGGCGCGACGCTTATCCGACCAACATATCATAACGGAGTGATCGCATTGAACATGCCAATGACCGAAAGAATAAGAGCAGGCAAGCTATTTACC
TTACAGCCTACGCCGCGCTGCGAATAGGCTGGTTGTATAGTATTGCCTCACTAGCGTAACTTGTACGGTTACTGGCTTTCTTATTCTCGTCCGTTCGATAAATGG
20
AspMetCysGluGlyLeuProGluLysArg
MspI
1500
GATATGTGCGAAGGCTTACCGGAAAAAAGA
CTATACACGCTTCCGAATGGCCTTTTTTCT

Fig. 3 Nucleotide sequence from the *Eco*RI site at the end of the *lacZ* gene to the beginning of the *lacA* gene and the amino acid sequence deduced for the *lac* permease. Nucleotides are continuously numbered, amino acids according to their position in the protein. Restriction endonuclease sites are also shown. The positions of *Msp*I, *Hha*I, *Hae*III and *Mbo*II cuts were derived from the restriction map; *Sau*3A, *Alu*I and *Taq*I cuts were deduced from the DNA sequence.

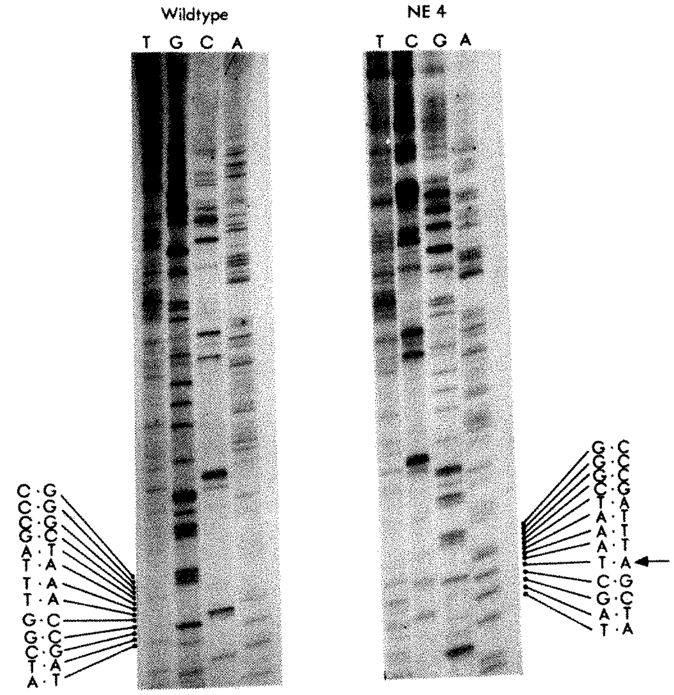

Fig. 4 Autoradiographs of sequencing gels according to Sanger *et al.*[12]. The sequence of the region carrying the NE4 mutation in which the Trp codon TGG no. 33 of the *lac* permease gene is changed into the amber triplet TAG is compared with the wild-type sequence of the same region.

gene into the amber triplet TAG. The sequence analysis of this mutation confirmed the suspected reading frame of the *lacY* gene and limited the possible start points. There are only three ATG and no GTG triplets preceding the NE4 amber codon. Only one of the ATG codons is preceded in a proper distance by a 'Shine–Dalgarno sequence'. Thus, we suspect that the *lacY* gene starts after an intergenic region of 45 nucleotides with an ATG codon and ends 1,251 base pairs later with a single ochre codon. The start codon is preceded at a distance of 8 base pairs by the sequence TAAGGA, which can hybridise to the 3′-end of the 16S ribosomal RNA HO-AUUCCU determined by Shine and Dalgarno[15]. The *lacY* gene recognition site for the 16S rRNA is two nucleotides longer than that of the *lacZ* gene, which is AGGA[16]. The sequences of the end of the *lacZ* gene and the beginning of the *lacY* gene have a high A and T content and show a palindromic symmetry. Figure 5*a* shows a possible secondary structure of the mRNA forming a stem from positions 47–87 and 155–118. The loop of 30 bases contains the 16S ribosomal RNA recognition site and the AUG start codon of the *lacY* gene. Similar secondary structures were proposed by Selker and Yanofsky[17] for *trpB*, *trpE*, *trpG* and *galT* as well as for *lacI*.

The nucleotide sequence from base pair 1,426 to 1,500 (Fig. 3) is compatible with the first 25 amino acids of transacetylase determined by Zabin and Fowler[18]. The start codon for fMet-tRNA cannot be unambiguously deduced from the DNA sequence. An interesting possibility is the TTG codon preceding the AAC asparagine codon. TTG has been reported only as a start codon of a restart fragment of the *lac* repressor[19,20]. A less likely possibility is the GTG codon which lies four triplets upstream of the AAC coding for the N-terminal asparagine. No possible secondary structure of the mRNA could be seen. A ribosomal binding site precedes the UUG in a distance of three bases. The sequence UAACGGAGUGAUC is able to form—with interruptions—of several base pairs with the 3′-end of the 16S ribosomal RNA (Fig. 5*b*).

The sequence of the *lacY* gene as well as the preceding ribosomal binding site carried by the 3,500-base pair *Eco*RI-*Bgl*II fragment cloned without any selection for expression proved to be identical to that of the 2,100-base pair fragment. Thus, we can exclude the possibility that we isolated a *lacY* mutation in our first isolation procedure. We have also cloned the 2,100-base pair fragment in the multicopy plasmid pUR2 (ref. 21). On this plasmid the *lacY* gene is expressed under the control of the *lac* promoter. Strains containing this hybrid plasmid showed at least a fivefold increase in permease activity compared with a strain with the *Y* gene on the F′-*lac pro* episome (P. Betteridge, personal communication). Thus, competition of permease and *M13* coat protein mRNA for a limiting factor or competition of the proteins themselves for space in the bacterial membrane are possible explanations for the low permease activity in strains harbouring the phage *M13*mp2561.

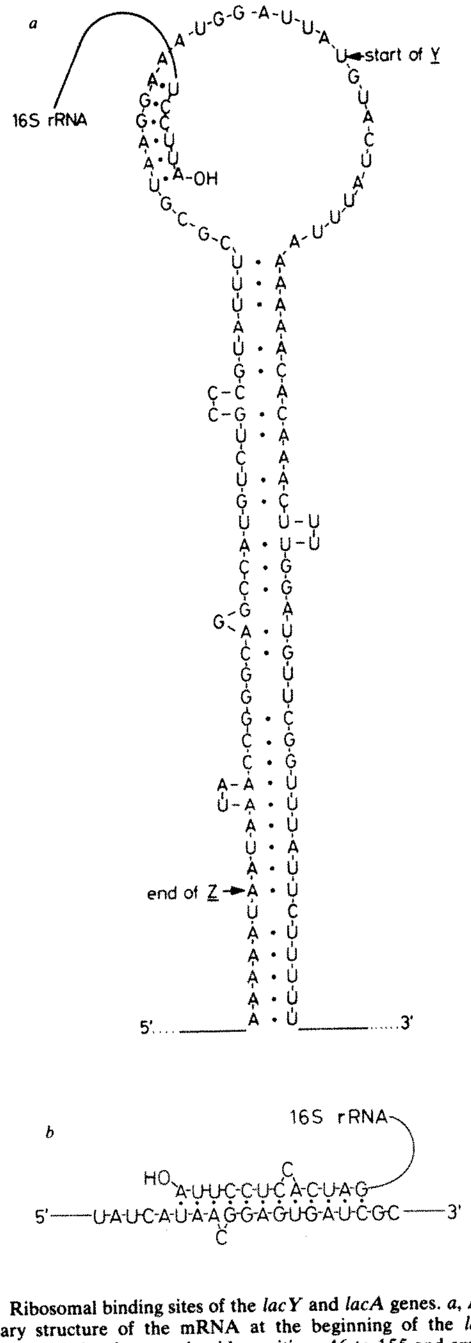
a
16S rRNA
A-OH
start of Y
end of Z
5′
3′
b
16S rRNA
HO A-U-U-C-C-U-C-A-C-U-A-G
5′—U-A-U-C-A-U-A-A-G-G-A-G-U-G-A-U-C-G-C—3′

Fig. 5 Ribosomal binding sites of the *lacY* and *lacA* genes. *a*, A possible secondary structure of the mRNA at the beginning of the *lacY* gene spanning the region from nucleotide positions 46 to 155 and exposing the ribosomal binding site and the AUG start codon in the loop. *b*, Possible extent of hybridisation of the 3′-end of the 16S ribosomal RNA to the mRNA at the beginning of the *lacA* gene.

Protein sequence of *lac* permease deduced from the DNA sequence

Ehring *et al.* (see accompanying article)[4] found that *lac* permease synthesised *in vivo* and *in vitro* started with the N-terminal amino acids predicted for native permease from the DNA sequence. The DNA sequence suggests that it consists of 417 amino acids (Fig. 3), resulting in a molecular weight of 46,504. This is higher than the 29,000 reported by Jones and Kennedy[22] and the 30,000 reported by Teather *et al.*[3]. A similar size was found by Ehring *et al.*[4] for permease synthesised *in vivo* and *in vitro*. Thus, either the membrane protein *lac* permease behaves atypically on SDS gels or it is proteolytically degraded at its C-terminus.

lac permease is a strongly basic protein of low polarity (29% polar, 71% unpolar); the high content of phenylalanine residues, 56 out of 417, is remarkable. Of these, 22 are clustered in the N-terminal region, making it highly hydrophobic. The first N-terminal lipophilic part of the protein spans 26 amino acids at positions 9–34 and has a hydrophobicity index of 4.0 determined according to Segrest and Feldmann[23]. It is followed in short distances by three additional hydrophobic sequences at positions 45–66, 76–99 and 108–118, with hydrophobicity values of 2.5, 3.4 and 2.4, respectively. The middle part has a more hydrophilic character, containing charged and less hydrophobic amino acids. This region, from residue 119 to 274, is separated into two parts by a lipophilic sequence of 11 amino acids (177–187), with a hydrophobicity index of 3.3. The C-terminus again contains hydrophobic sequences in clusters: residues 275–283, 291–299, 326–334, 347–357 and 381–395, with corresponding hydrophobicity indices of 3.7, 2.3, 2.7, 3.0 and 2.7.

We conclude that we can divide lactose permease into four structural parts: a hydrophobic N-terminus, two hydrophilic regions in the middle part of the protein, separated by a lipophilic sequence of 11 amino acids, and a hydrophobic C-terminus. We propose that the hydrophobic regions at the N-terminus and the C-terminus function as anchors by which the protein is fixed in the membrane; they could also form pores for galactoside and proton transport. The length of the lipophilic sequence from residue 177 to 187 separating the two hydrophilic parts may be sufficient to penetrate the membrane. Thus, the function of sugar recognition and binding may be carried out by the two hydrophilic domains which could occupy sites inside and outside the membrane. Genetic and biochemical data support the idea that *lac* permease is active as an oligomer, possibly as a dimer[24].

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Structure of the junction between communicating cells

P. N. T. Unwin[*] & G. Zampighi[†]

[*] MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, UK
[†] Department of Anatomy, Duke University Medical Center, Durham, North Carolina 27710

An 18-Å resolution map of the 'gap junction' has been obtained by electron microscopy. The protein oligomer in the junctional membranes, the 'connexon', is a cylinder composed of six subunits which are tilted around its axis. Analysis of two different subunit configurations suggests how the connexon might regulate the passage of small molecules between cell interiors.

SPECIALISED regions of contact known as gap junctions[1] exist between apposed plasma membranes of communicating cells. These junctions are believed to mediate and regulate the passage of ions and small molecules between the cell interiors[2]. They are composed of units, called connexons[3], which are embedded in the apposed membranes, in register, and linked to each other. The connexon is often organised on a regular hexagonal lattice, as first described in detail by Robertson[4]. Its structure in this lattice has been studied by electron microscopy[5,6] and low angle X-ray diffraction[7,8]. It is thought to be constructed from protein oligomers arranged in the form of an annulus and delineating a narrow channel through the membrane. This channel is presumed to provide the regulated hydrophilic pathway connecting the cytoplasms of adjacent cells.

Experiments with fluorescent tracer molecules (see, for example, refs 9, 10) suggest that the channel can accommodate a wide range of molecular sizes (up to about 800 daltons in mammalian cells[10]). Cytoplasmic free Ca^{2+} concentration[11] and/or pH (ref. 12) may be involved in regulating its permeability. Although electron microscopic and X-ray evidence point to changes in the size[13], shape and packing of the connexon[8,13], it is

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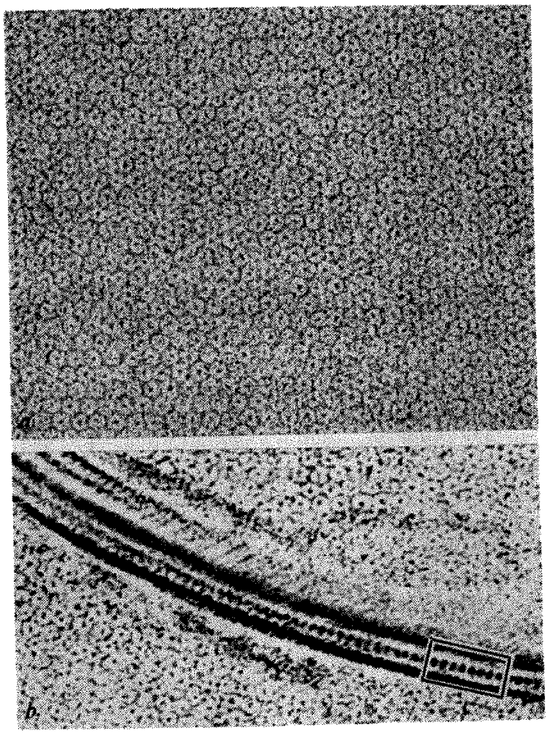

Fig. 1 Electron micrographs of isolated gap junction sheets viewed from a direction perpendicular to the plane of the membranes (*a*) and a direction more nearly parallel to this plane (*b*). In *a* the connexons are seen as annular units organised on a hexagonal lattice, with unit cell dimension 85 Å; in *b* they are seen as bridges connecting the two apposed membranes. The view in *b* arises from a fortuitous curling up of a sheet at its edge; the region outlined (looking along the [1, 1] direction) was used to estimate the average density profile in the direction perpendicular to the plane of the junction, and hence the variation in amplitude and phase along the lattice line through the origin. Uranyl acetate negative stain. ×300,000.

not clear how changes in the structure cause the permeability changes.

We have studied the structure of isolated gap junctions to a resolution of 18 Å by electron microscopy of specimens tilted at various angles to the incident beam. The three-dimensional maps reveal the configuration of the connexon subunits, their relationship to the membrane surfaces and the nature of the region they enclose, within the membrane. The subunits are found to take up two alternative configurations; the differences between them suggest a simple molecular mechanism by which the permeability may be controlled *in vivo*.

Electron microscopy

We used rat hepatocyte junctions, isolated and negatively stained as described previously[14]. *En face* views show the (superimposed) connexons as annuli arranged on a hexagonal lattice of unit cell dimension 85 Å (Fig. 1*a*). Edge-on views show the connexons as links which bridge the extracellular space between the apposed membranes, creating a gap of 30–40 Å (Fig. 1*b*). These junctions seem to be the same as those isolated by others (for example, ref. 15). They can be converted to a second form having the same hexagonal lattice, but in which individual connexons are less distinct, by dialysing them against water at 4° C for several days[14]. We shall describe below how the normal form, as in Fig. 1, and this second form differ in their three-dimensional structure.

We investigated the structure of the normal junctions by Fourier methods[14,16,17]. Specimens were tilted at angles of between 0° and 79° to the incident 100-kV electron beam, and micrographs were recorded using low electron doses to minimise the effects of radiation damage. Densitometry and computer processing were carried out on selected micrographs[14] from several specimens to give the Fourier terms into which the structure can be analysed. The three-dimensional Fourier transform consists of a set of reciprocal lattice lines, perpendicular to the plane of the junction, along which the amplitudes and phases vary continuously. The Fourier terms determined from micrographs taken with different tilts give the values of the amplitudes and phases at different sections through these lattice lines. To map out the continuous variation along each line, the terms were combined, in the first instance, according to the lowest symmetry compatible with the hexagonal projected structure, that of the crystallographic space group, P3. We began with data from specimens recorded with smallest tilt, and only compared values which along any given line lay within 0.0025 Å^{-1} of each other. Twenty-six micrographs were used, each providing up to 30 independent measurements of amplitude and phase. The average phase error, based on comparisons between each new measurement and all previously accumulated phases related by symmetry, was 21°. Smooth curves, then drawn through the combined data, demonstrated that points equidistant from the midpoint along each lattice line had equivalent amplitudes and opposite phases. This result indicated that, at least to the resolution of the analysis, the junction had the higher symmetry of the space group, P6. Therefore, to improve the accuracy, the data were refined a second time according to this latter space group.

The amplitude and phase curves (Fig. 2) show minor departures from the symmetry of the space group, P622, relating the two oppositely facing halves of the junction by dyad axes lying in its central plane. Therefore, the two halves are almost, but not exactly, equivalent. The departures from perfect symmetry were probably produced as the stain dried around the specimen and support film. In favour of this argument, we found that the phase error introduced in combining data from different junctions depended on whether we took the surface next to the support film to be at the top or bottom of the structure. In all cases, the assumption of a consistent location for the support film gave the smallest value for this error.

It was useful, although not critical for an understanding of the structure, to estimate the average density profile in the direction perpendicular to the plane of the junction, and hence the variation in amplitude and phase along the lattice line through the origin (the 0,0 lattice line). Edge-on views of the junction, as seen in Fig. 1*b*, were analysed for this purpose. Fourier transforms calculated from these views gave directly the variations along the 0,0 and 1,1 lattice lines. Correlation with the 1,1 lattice line derived from the combined data then allowed us to select the most accurate 0,0 lattice line and determine the scale for the variation along it. Its peak amplitude was found to be six times that of the 1,1. We assumed that the profile corresponding to this variation was reasonably correct because it could be closely matched to features in a preliminary three-dimensional map obtained without the 0,0 lattice line included in the calculations.

The complete transform consisted of 11 crystallographically independent lattice lines (0,0; 0,1; 0,2; 0,3; 0,4; 1,1; 1,2; 1,3; 2,1; 2,2; 3,1), and extended in all directions to a resolution of about 18 Å. We collected 192 Fourier terms by sampling the smooth curves at intervals of 0.0025 Å^{-1} along these lines.

Three-dimensional map

The map calculated by Fourier synthesis of these terms depicts the membrane surfaces and the hydrophilic surfaces of the connexon subunits, as these are the parts of the structure exposed to the negative stain. In Fig. 3 we consider one half of this map, corresponding to one of the two oppositely facing halves of the junction. There is a rather featureless zone, presumed to be the membrane, with matter on either side

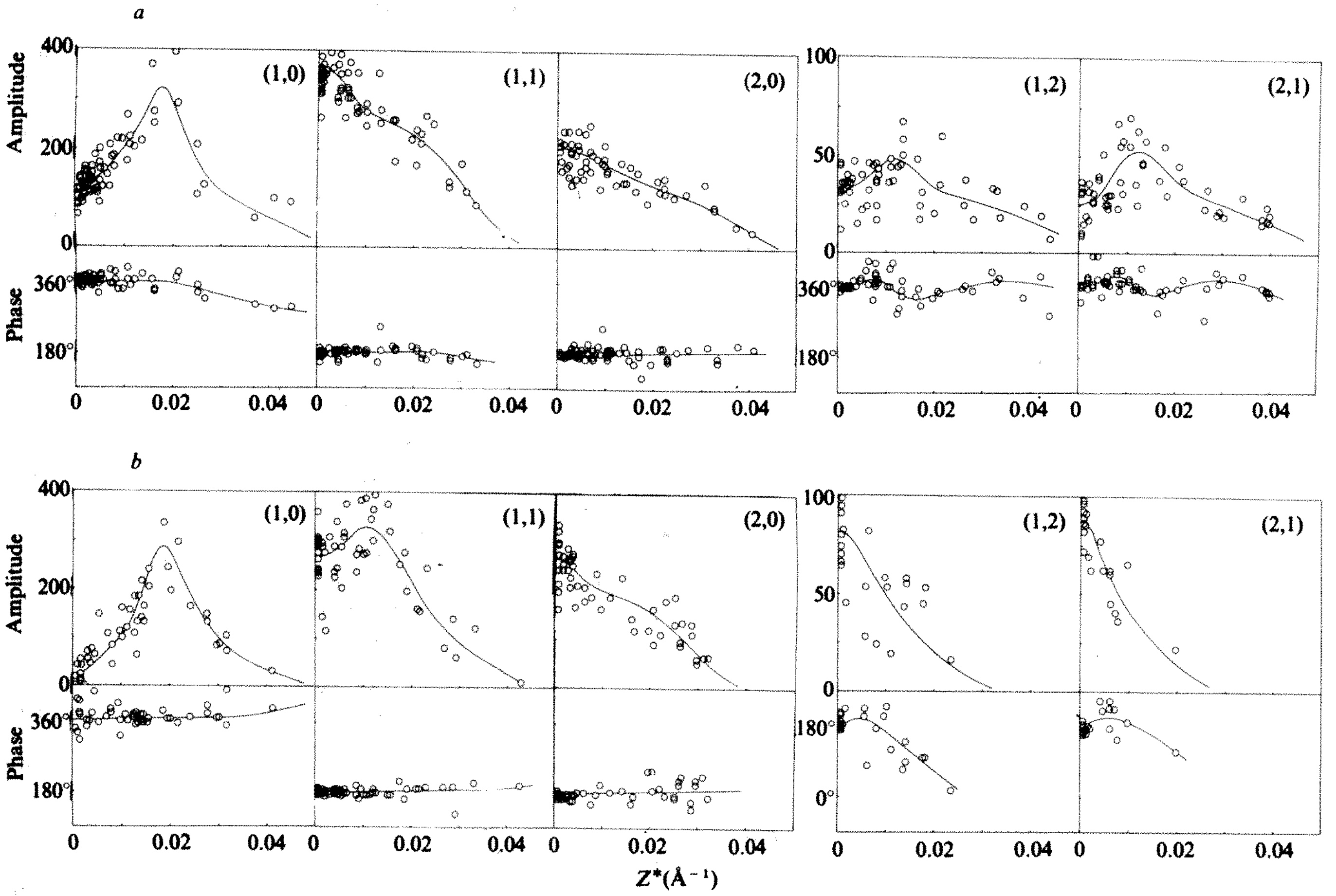

Fig. 2 Values of amplitude and phase determined along reciprocal lattice lines from micrographs of specimens tilted to the incident electron beam by angles up to 79°; *a*, the normal junction (as in Fig. 1); *b*, the form after the transition. Z^* is the distance from the midpoint along the lattice line. The tilt range 0–55° was obtained conventionally, using a goniometer stage; the additional tilt to 79° was achieved by bending the support grids. The data have been combined assuming the space group, P6; thus, the Friedel relationship gives the values for negative Z^*. Smooth curves drawn through the points and sampled at intervals of 0.0025 Å^{-1} gave the Fourier terms from which the maps were calculated. The data extend to the points where the amplitudes became comparable with the local background level, that is, to a resolution of ~18 Å in *a* and ~23 Å in *b*. The high tilt angles (>60°) were required to determine the variation along the 1,0 lattice line at values of $Z^* \geq 0.02\ \text{Å}^{-1}$; only the strong low resolution lattice lines (1,0; 1,1; 2,0) were used in the refinement of these data. The additional lattice lines, obtained for *a*, but not shown, are weak and do not significantly affect the detail in Fig. 4*a*; they improve the definition in Fig. 3, but do not contribute new features.

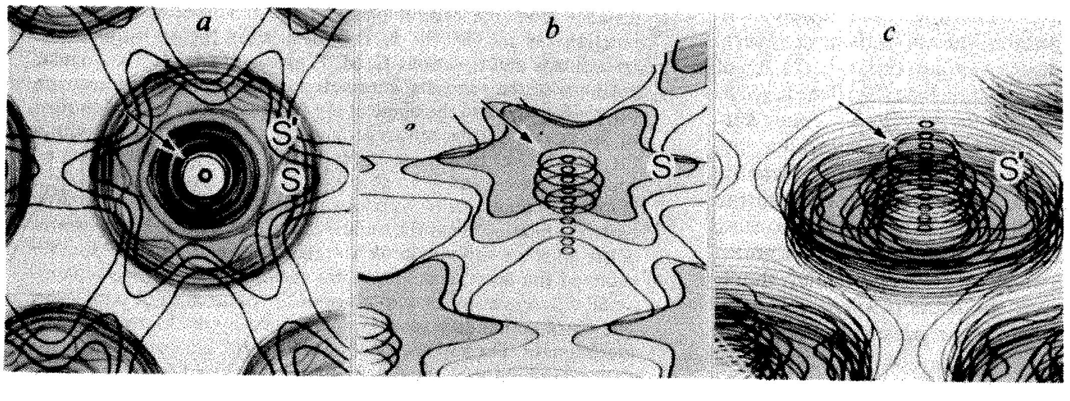

Fig. 3 Map of one half of the gap junction, as seen from the interior of the cell. Sections through the structure were traced onto Perspex sheets, which were then stacked on top of each other; the complete map (*a*) is composed of a portion (*b*) containing the cytoplasmic face, stacked on an equal portion (*c*) containing the extracellular face. The orthogonal view (*a*) emphasises changes in the angular disposition of the subunits; the oblique views (*b*, *c*) emphasise the subunit features in relation to the membrane surfaces. The contours identify the stain-excluding regions formed by the components of the junction (compare Fig. 4*a*); the exact positions of their boundaries are not accurately determined because these are sensitive to small errors in the variation along the 0,0 lattice line. The detail protruding from the membrane is emphasised by shading and by additional contours. The arrow points to the opening surrounded by the connexon subunits, one of which is labelled, S and S'. Hexagons have been drawn at the 6-fold axis of the connexon on each of the Perspex sheets. The half of the junction not shown contains essentially the same detail, but is slightly less well contrasted by the stain.

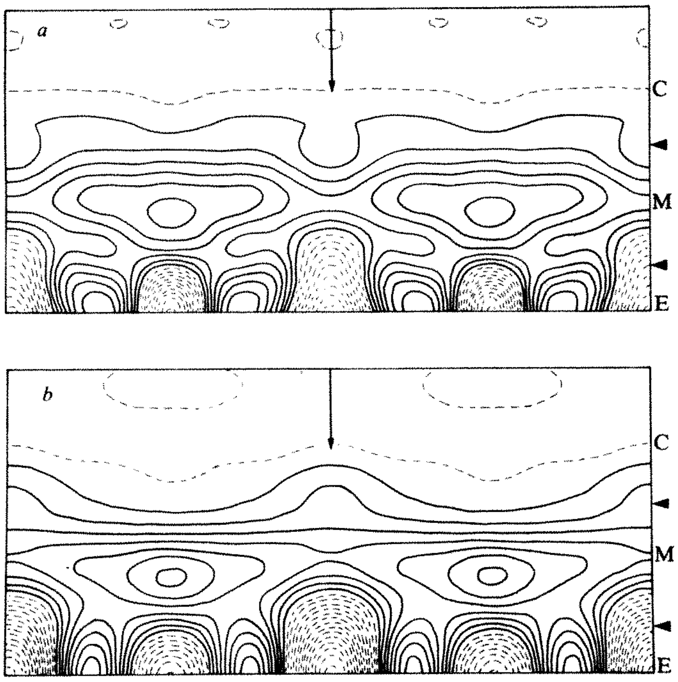

Fig. 4 Sections through the maps perpendicular to the plane of the junction, along a line joining the connexon axes; *a*, the normal junction (as in Fig. 3); *b*, the form after the transition. Arrowheads point to the approximate locations of the cytoplasmic (upper) and extracellular (lower) membrane surfaces. The zones C, M and E refer to the cytoplasm, membrane and extracellular space, respectively. Matter at the cytoplasmic surface moves towards the connexon axis (vertical arrow), closing the opening there, in going from *a* to *b*. This effect is contributed mainly by the change in amplitude along the strong 1,1 lattice line. Contours corresponding to stain-excluding regions (negative contours) are drawn as continuous lines; contours corresponding to stain-filled regions (zero and positive contours) are drawn as broken lines. The same Fourier terms along the 0,0 lattice line were used in the calculations for both maps. The sections contain two unit cells in the horizontal direction and half of the junction in the vertical direction. The absolute scale in the vertical direction is sensitive to flattening effects associated with the stain drying around the specimen[22]. Assuming a total thickness of 50 Å for the membrane[8], the connexon must extend about 75 Å from the central plane of the junction.

(shaded in Fig. 3) clustered around the 6-fold axes. The clusters are hexamers, presumed to be the exposed portions of six connexon subunits protruding from the cytoplasmic and extracellular faces of the membrane. The subunits have several features of interest. Their length is approximately 75 Å, of which less protrudes from the cytoplasmic face (5–10 Å; Fig. 3*b*) than from the extracellular face (15–20 Å; Fig. 3*c*). They surround a narrow opening (arrow) which penetrates the membrane and widens to a diameter of about 20 Å in the extracellular region of the junction. They are centred on the cytoplasmic face at points (such as S) where neighbouring connexons come closest, and on the extracellular face at points (such as S′) in between these locations. The subunits are therefore at different angular positions about the 6-fold axes on the two faces and must be inclined in their passage through the membrane.

The distinct annular appearance of the connexon in the micrograph (Fig. 1*a*) can now be understood to result from summation of annular features on both the cytoplasmic and extracellular faces. The 6-fold modulations around this annulus are weak, because the modulations at the cytoplasmic and extracellular faces (peaked at S and S′) are not in register and to some extent cancel each other out.

Two quaternary states

We investigated the structure of the second form of junction, produced by dialysis. Data were collected and combined from 20 micrographs of specimens tilted through the same range of angles as before; the average phase error, assessed in the same way, was 22°. Figure 2 compares the variations in amplitudes and phases for the two forms. There are large differences along the 1,2 and 2,1 lattice lines where the amplitudes peak at different places and the phases differ by about 180°. There are smaller differences, involving mainly amplitudes, along others. Low angle X-ray diffraction patterns from orientated pellets of isolated gap junctions[7,8] corroborate the curves obtained for the normal junction (Fig. 2*a*). The first four X-ray lattice lines (0,1; 0,2; 1,1; '1,2') are the strongest, and the 1,2 lattice line is peaked away from the equator. The most pronounced changes that the transition should produce in the X-ray pattern are a convergence of the off-axial peaks on the 1,2 lattice line into a single peak on the equator, and a splitting of the equatorial peak on the 1,1 lattice line into two slightly off-axial ones.

The maps of the junctions in the two states show several differences. These were interpreted in terms of two kinds of displacement of the subunits, one radial and the other tangential about the 6-fold axis through the centre of the connexon. The radial displacement is insignificant near the centre of the junction, but is pronounced near the cytoplasmic face (Fig. 4). The opening delineated there by the subunits (arrow in Fig. 4*a*) disappears after the transition (Fig. 4*b*), indicating movement of matter towards the connexon axis. A radial displacement of the order of 5 Å would be consistent with the maps, but we emphasise that the method of visualising the structure, using

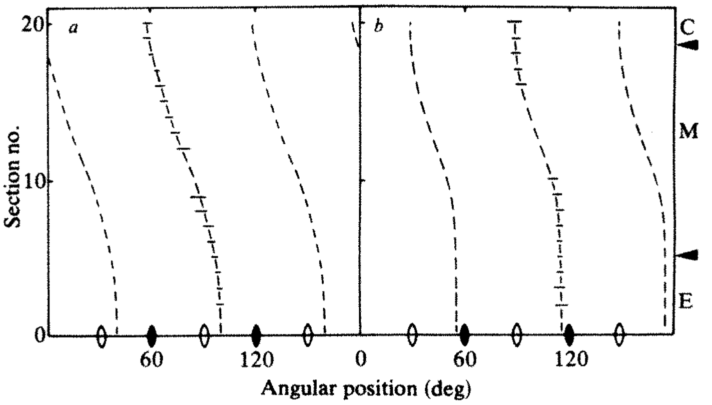

Fig. 5 Two-dimensional net showing the angular positions of the subunits about the 6-fold axis of the connexon in relation to distance from the central plane of the junction; *a*, the normal junction (as in Fig. 3); *b*, the form after the transition. The arrowheads and notations C, M, E are as in Fig. 4. The angular positions of the subunits were determined from sections through the maps parallel to the plane of the junction. They were identified by the peaks in the contours around the connexon axis, or (within the membrane) by the locations where the hexagonally shaped contours came nearest to this axis. An indication of the errors involved in these estimates is given by the lengths of the horizontal bars. Modulations near the middle of the membrane and the centre of the junction were too weak for reliable estimates to be made; we assumed the most direct paths for the subunits in these regions. Small differences in the curvature of the paths are not significant. The absolute handedness was determined by the known sense of specimen tilt. The variations along the 1,2 and 2,1 lattice lines have a strong influence on the details shown. The dyad symbols refer to putative dyad axes lying in the central plane of the junction along the [1,0] (filled symbols) and [1,1] (open symbols) crystallographic directions, and relating the apposed connexons facing each other in the two membranes. Perfect dyads are not present because the two halves of the junction are not quite equally contrasted by the stain. The positions of the subunits in the more poorly stained halves are consistent with the presence of dyads.

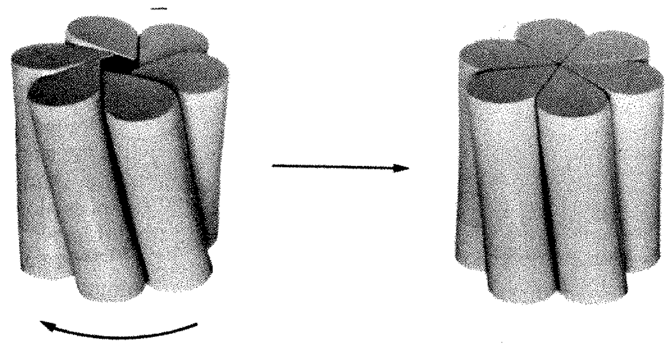

Fig. 6 Wooden model of the connexon, depicting the transition from the 'open' to the 'closed' configuration, as suggested by the maps. It is proposed that the closure on the cytoplasmic face (uppermost) is achieved by the subunits sliding against each other, decreasing their inclination and hence rotating, in a clockwise sense, at the base. The darker shading on the side of the model indicates the portion which would be embedded in the membrane. The subunit inclinations depicted are the average inclinations estimated from the nets (Fig. 5), and the diameter at the base of the connexon, as indicated by our data, is fixed. The radial displacement of each subunit at the cytoplasmic end would be about 6 Å, given the observed inclination change of 5° distributed over its 75-Å length.

negative stain, does not permit an accurate estimate to be made. This redistribution of matter on the cytoplasmic face accounts for the change in contrast of the connexon seen in the micrographs[14]. The annular appearance of the connexon would become less distinct after the transition because the detail on the cytoplasmic face no longer superimposes to reinforce the annular detail on the extracellular face.

The tangential displacement about the connexon axis was measured by tracing the paths followed by the subunits from the two maps. Two-dimensional nets were plotted giving the angular positions of the subunits in relation to distance from the central plane of the junction (Fig. 5). The nets show that the subunits follow fairly straight, slightly sigmoidal, paths which are differently inclined in the two structures. Their average inclination is reduced by the transition (made more nearly perpendicular to the plane of the junction) from 14° (Fig. 5*a*) to 9° (Fig. 5*b*)—a total change of about 5°. In addition to this tilting of the subunits, the connexon itself rotates by about 23° within the crystal lattice. More subtle effects, for example, distortions of the subunits, may be present but are not resolved. It would be difficult to detect small changes in the lengths of the subunits[8] by our technique.

The inclination changes produce rotations between equivalent parts of apposed connexons (that is, connexons facing each other in the two membranes) about their common 6-fold axis. The magnitude of these rotations can be estimated from Fig. 5, as the two halves of the junction are related to each other crystallographically (see Fig. 5 legend). The maximum value, 28°, is in the central plane of the junction, and here the subunits of apposed connexons must slide over each other by a distance of about 11 Å.

Structure of the connexon

The features in the three-dimensional map (Fig. 3) indicate that the connexon is an annular oligomer composed of six protein subunits which span the membrane and protrude from either side. Assuming a value of 25–30,000 daltons (refs 14, 18–20) for the molecular weight of the gap junction protein and 1.3 $Å^3$ per dalton for the partial specific volume (the value obtained for another, well studied, membrane protein[17]), each subunit must occupy a volume of at least 32,500 $Å^3$. To fill this volume, its cross-section inside the membrane would have to be at least equal to that outside. We suppose, then, that the subunits are roughly rod-shaped, about 25 Å in diameter and 75 Å long. A picture similar to this was deduced from a combined study by electron microscopy and low angle X-ray diffraction[8]. In addition, our analysis shows a variation in the dimensions of the central opening delineated by the subunits. It is about 20 Å wide in the extracellular region of the junction, but is narrower within the membrane where it is not fully resolved. The path taken by the subunits is also inclined tangentially with respect to the 6-fold axis of the connexon, giving the whole assembly a left-hand character.

The transition to the second configuration of the connexon is produced by a radial inwards motion of the subunits near their cytoplasmic extremities and a reduction of their inclination tangential to the 6-fold axis. These movements are accompanied by a rotation between the subunits of apposed connexons in the central plane of the junction. A comparable rotational displacement, together with tilting of the subunits, occurs between the two layers of the protein disk of tobacco mosaic virus when it undergoes a transition into the helical assembly found in the virus[21].

We note that radial motion at one end of the connexon could be transmitted through a change of subunit inclination into rotational motion at the other end by a process in which the subunits slide over each other along their lines of contact. The small displacements involved could be accommodated by only very minor distortions of the subunits. Such an action, which is depicted in Fig. 6, seems to describe well the changes in configuration suggested by our maps. The radial displacement of each subunit at the cytoplasmic end should be ~6 Å, given the observed change of inclination of 5°, distributed over its length. Displacement by about this amount is suggested by the details in Fig. 4.

A rearrangement of essentially rigid subunits has not so far been found in oligomeric membrane proteins, but is a common feature of soluble multiple subunit, multi-state assemblies which change their structure and function in response to chemical stimuli. The particular rearrangement in Fig. 6 provides a simple mechanism for opening and closing a narrow channel through a membrane. This mechanism may be the one by which the gap junction regulates the passage of small molecules between living cells. The dimensional changes seem to be of the correct magnitude to account for the observed permeability changes.

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LETTERS

IR flashes from the X-ray rapid burster

A. W. Jones, M. J. Selby, C. M. Mountain & R. Wade

Astronomy Group, Blackett Laboratory, Imperial College, London SW7 2BZ, UK

C. Sánchez Magro & Mercedes Prieto Muñoz

Instituto de Astrofísica de Canarias, Universidad de La Laguna, Teberife Spain

We confirm here the report of Kulkarni *et al.*[1] who discovered IR bursts from the X-ray rapid burster, MXB1730−335. We observed two bursts at 2.2 μm, each of energy ~10^{31} J, and a new feature; several bright, brief flashes ($\Delta t < 1$ s) superimposed on the burst continuum. The brevity, luminosity and inferred brightness temperature of the flashes ($T_B \geq 10^{10}$ K) imply a powerful, non-thermal emission mechanism. Simultaneous observations across the whole spectrum with high time resolution would be invaluable in understanding the emission process.

The rapid burster was discovered in 1976 by Lewin *et al.*[2] at MIT and is unique in producing ~10^3 X-ray bursts a day in its active periods. These periods occur about every 6 months and last 2–6 weeks[3]. Liller[4], and Kleinmann *et al.*[5] have shown that the source lies in a compact globular cluster, now called Liller 1, at a distance of 10 kpc. By 1978 it had become clear that the same cluster also produces 'special' bursts[6], similar to those from the more usual X-ray burst sources. They are characterised by a softening of the X-ray spectrum during decay and are now known as Type I bursts. The rapid bursts are classified as Type II. In April 1979 Kulkarni *et al.*[1], observing from Kavalur in India, detected six bursts at 1.6 μm which they attributed to the Type I X-ray bursts.

On 8 August 1979 Type II bursts were seen by the X-ray satellite Hakucho[7], heralding a new period of activity. We attempted IR observations in early September using the 1.5 m IR flux collector at Izaña, Tenerife. The detector was an indium antimonide diode cooled to 75 K. The cooled filter had an effective wavelength of 2.2 μm and a bandwidth of 0.4 μm. We used a two-mirror focal plane sky chopper[8] at 20 Hz, with one beam (20 arc s diameter) centred on Liller 1 and the reference beam 20 arc s to the north. For calibration we used ϕ Ophiuchi ($m_K = 2.27$) taking its flux as $F_\lambda = 4.8 \times 10^{-11}$ W m^{-2} μm^{-1} in accordance with Johnson's[9] absolute flux calibration.

Table 1 lists the dates and times of our observing periods; a total of 5 h 15 min. In this time we saw only two bursts, 1 min apart, on 5 September 1979 (Kulkarni *et al.* saw six in 2.5 h). The chart recording of the bursts is shown in Fig. 1. They appeared at 21 h 04 min 01 s and 21 h 04 min 55 s UT ± 1 s, and lasted 20 s and 10 s respectively. They are similar in shape to the Kavalur bursts, except for the seven sharp spikes accompanying them. Our nominal time resolution, set by the output filter of our phase-sensitive detector (PSD) was 0.3 s, but the widths of two of the spikes (1d and 2c) remain unresolved (see Table 2). The negative-going spike, marked A in Fig. 1, is probably an artefact of the PSD caused by a sudden, rapidly increasing signal. We have been able to reproduce this feature with a laboratory PSD. The other spikes do not seem to be spurious. They appear nowhere else on the chart trace and are closely associated with the structure of the bursts. Assuming they are real, we can properly describe them as flashes.

Table 1 IR observations of the rapid burster

Date (September 1979)	Times (UT) Start (h min)	Finish (h min)	Duration (h min)	No. of bursts
5	20 50	22 10	1 20	2
6	20 20	22 05	1 45	0
11	21 10	21 45	0 35	0
12	20 05	21 40	1 35	0
		Total	5 15	2

In Table 2 we list some observed and derived characteristics of the flashes assuming a distance of 10 kpc and an interstellar absorption of 1.1 mag (ref. 5). With the data from Table 2 and Fig. 1 we can make the following observations.

Why are IR bursts visible at all? The MIT group[10] have measured the spectra of several X-ray bursts, both Type I and Type II, and have fitted them to black-body curves of temperature $T \approx 2 \times 10^7$ K. The inferred radius of the X-ray emitting region is $R \approx 10^4$ m. If we extrapolate the black-body spectrum into the IR we would expect a flux $F_\lambda \sim 10^{-23}$ W m^{-2} μm^{-1} at 2.2 μm; a factor of 10^{11} below our observed value.

Two of the flashes are unresolved at our instrumental time resolution of 0.3 s, putting an upper limit on the size of the emitting region of $R = 10^8$ m. This implies an IR brightness temperature $T_B \geq 4 \times 10^{10}$ K. If we assume that the IR radiation is coming from the same small region as the X rays, T_B rises to an improbable 4×10^{18} K.

If the radiation is isotropic, then the IR burst energies in our 0.4-μm bandwidth are 9×10^{30} J and 6×10^{30} J, and the peak luminosity is 2.2×10^{30} W. This compares with ~10^{32} J and ~10^{31} W for the Type I X-ray bursts[3].

It may be significant that the three flashes in each burst occur at similar times after the initial rise (see Table 2), except

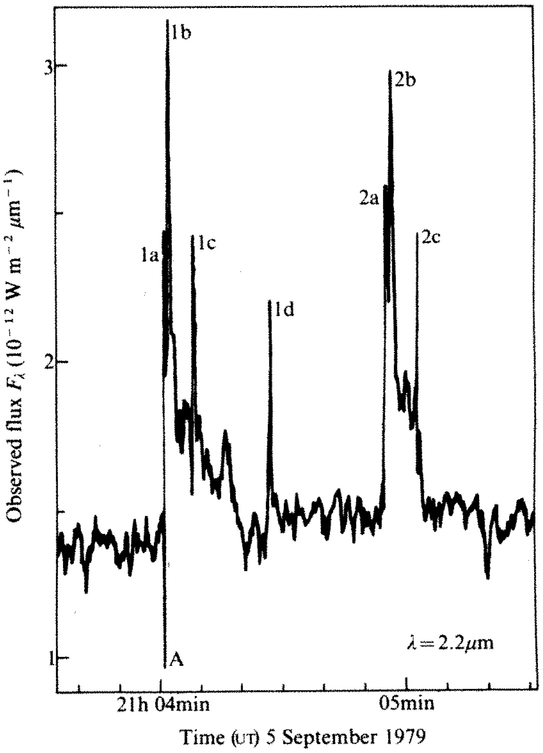

Fig. 1 Two IR bursts from the X-ray rapid burster, accompanied by seven brief flashes. The flux scale is not corrected for interstellar absorption. The feature at A is an artefact of the detection system.

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Table 2 Details of the IR flashes

Flash no.	Time of peak (s ± 0.1)	Half-peak width (s ± 0.1)	In bandwidth 0.4 μm Luminosity (10^{30} W)	In bandwidth 0.4 μm Energy (10^{30} J)
1a	0.0	0.5	1.3	0.6
1b	1.2	0.9	2.2	1.9
1c	7.0	0.8	1.3	1.0
1d	25.7	0.3	1.0	0.3
2a	54.2	0.6*	1.4	0.8
2b	55.6	1.5	1.9	2.8
2c	61.7	<0.3	1.2	<0.4

The half-peak widths, luminosities and energies were measured with respect to the background on either side of each burst. Isotropic radiation is assumed.

*This is the rise time: the half-peak width cannot be measured.

for the solitary flash 1d, which occurs in the space between the bursts.

Although we observed for 5 h 15 min the bursts appeared in the space of 1 min, suggesting that they may be physically associated. Kulkarni *et al.* noted similar groupings among some, but not all, of their bursts.

There are no clear spikes in the Kavalur bursts, but they did have a lower time resolution (0.6 s) and the signals were 2 or 3 times noisier than ours. It is not clear whether flashes would have been detected if they were there. The difference in wavelength could also be important. If the spectral index of the flashes is different from that of the burst continuum, then the flashes may be apparent at one wavelength but not at another.

We do not intend to go into the details of possible mechanisms, but we conclude: (1) As there have been no simultaneous X-ray/IR observations, we cannot say for certain that our bursts correspond to X-ray bursts; Type I X-ray bursts recur at intervals of hours, while the Type II bursts repeat at intervals of seconds or minutes. The IR bursts observed so far do not fit either category.

(2) If our observations do correspond to X-ray bursts, then the high brightness temperature and unexpectedly intense IR flux strongly suggest a non-thermal emission mechanism. The favoured explanation of the Type I X-ray bursts is a thermonuclear helium flash in accreted material on a neutron star[3,11]. This accounts for the observed X-ray behaviour very well, but does not predict an observable IR flux. Apparao and Chitre[12] propose that the IR bursts are produced by a 'cyclotron maser' operating above the poles of a magnetised neutron star in a close binary system.

Further observations at IR wavelengths should attempt time resolutions of <0.1 s to detect and resolve the flashes. Simultaneous observations at X-ray, optical and several IR wavelengths would be invaluable in understanding the emission process.

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Semi-empirical estimates of gravitational wave generation by stellar collapse

J. I. Katz

Department of Astronomy and IGPP, University of California,
Los Angeles, California 90024

The problems of the generation and detection of gravitational waves and the difficulty of detecting the burst of gravitational radiation produced by a stellar collapse in our Galaxy, or in neighbouring clusters of galaxies where such collapses are expected to occur frequently, have been reviewed elsewhere[1–3]. As stellar collapses, explosions, or supernovae are poorly understood, the values of the strength of these sources depend on uncertain assumptions. However, it is possible to make some independent estimates on semi-empirical grounds, using observed facts concerning the remnants of stellar evolution. These estimates, reported here, have serious weaknesses. They must involve oversimplified models and very crude standard calculations of the collapse and radiation processes. Also, because they are based on observed properties of stellar remnants, they say nothing about collapses which do not produce observable remnants. Although this selection may introduce a strong bias, these estimates deserve consideration because they are tied to real data.

Consider a simple model for the collapse of a stellar core of mass m into a dense remnant and expelled debris: ignoring the mass and momentum radiated away, the remnant has a mass $(1-\alpha)m$ and a velocity αv; the debris have a total mass αm and their barycentre has a velocity, in the opposite direction to that of the remnant, of $(1-\alpha)v$. If we assume that the debris cloud acts as a point at its barycentre, and that acceleration of remnant and debris occurs over a time t, then it is easy to estimate the emitted power P, energy E, and efficiency ε. The quadrupole moment is given by

$$Q \approx Mv^2t^2\alpha(1-\alpha) \tag{1a}$$

$$|\dddot{Q}| \approx Mv^2\alpha(1-\alpha)/t \tag{1b}$$

The duration of the radiation is also about t. Then

$$E \approx Pt \approx \frac{Gt}{5c^5}|\dddot{Q}|^2 \approx \frac{GM^2v^4}{5c^5t}\alpha^2(1-\alpha)^2 \tag{2a}$$

$$\varepsilon \equiv \frac{E}{Mc^2} \approx \frac{GMv^4\alpha^2(1-\alpha)^2}{5c^7t} \tag{2b}$$

Now we can apply our knowledge of certain classes of collapsed objects. For the X-ray sources in globular clusters a simple argument[4] based on the dynamical properties of the clusters shows that the recoil velocity of the remnant $\alpha v < 10^{-4}c$. For galactic pulsars $\alpha v \approx 10^{-3}c$. Taking $1-\alpha \approx 1$ and, with r the size of the exploding core, $t \approx r/v \approx (c/v)(10GM/c^3) \approx 10GM/vc^2$ gives

$$\varepsilon \approx \frac{1}{50}\left(\frac{v}{c}\right)^5\alpha^2 \tag{3}$$

Although we have an empirical indication of the recoil velocity αv, we must guess at v; take $v = 0.4c$. Then

Table 1 Angular momentum and efficiency of radiation of various objects

Object	l (cm^2 s^{-1})	ε
Neutron star rotating at breakup	10^{16}	10^{-4}
Crab pulsar $P = 0.03$ s	2×10^{14}	10^{-11}
Typical pulsar $P = 1$ s	6×10^{12}	10^{-17}
White dwarf $P = 71$ s	3×10^{16}	10^{-7}
White dwarf $P = 2\times10^3$ s	1×10^{15}	10^{-8}
White dwarf $P = 2$ h	3×10^{14}	10^{-10}

$$\varepsilon < 10^{-11} \quad \text{(globular cluster sources)} \tag{4a}$$

$$\varepsilon \approx 10^{-9} \quad \text{(galactic pulsars)} \tag{4b}$$

These estimates are disappointingly low. They also require very small values of α, in apparent disagreement with the observed masses of supernova debris. The low observed recoil velocities of the compact remnants imply that the core of the explosion is very symmetric; the moderate velocities and high masses of the exploded envelopes imply that the energy released in the core is spread over the massive envelope of the original star, slowing the velocity as a large mass is swept up. Our estimates yield kinetic energies of $<4\times10^{49}$ erg and $\approx4\times10^{50}$ erg from the globular cluster sources and the pulsars respectively, not far from observed supernova energies. The sweeping up of the stellar envelope occurs at much lower densities and on longer time scales than the core collapse, and hence radiates negligible gravitational radiation, despite the greater mass involved. An alternative estimate, assuming massive debris ($\alpha = \frac{1}{2}$), yields even lower values of ε than equation (4) because then the data require small values of v.

The preceding estimates have led to low values of ε because a model for the explosion was chosen in which any gravitational radiation producing asymmetry also produced a net recoil, which is strictly constrained by the data for the observed objects. There are other kinds of varying quadrupole which radiate, but which are not so constrained. Saenz and Shapiro[5] have calculated higher efficiencies from asymmetric core bounces. Another kind of asymmetry, resulting from the flattening of a collapsing rotating object, may be empirically estimated. We have no direct knowledge of the rate of rotation of the cores of ordinary stars before their collapse, but we do know how much angular momentum some of their compact remnants have; the observed spins of white dwarfs and neutron stars may be used as a guide. Accreting compact objects in binaries are observed to rapidly spin up (and, by indirect statistical arguments, must spin down when they are unobservable), so the present spins of neutron stars in X-ray binaries give no information about their spins at birth. The spin of one accreting binary white dwarf (DQ Her) is known to be 71 s. Because of its spin-up, the present period need not reflect the spin of the stellar core before it lost its envelope; nor do we know if the evolution of an accreting white dwarf which undergoes nova outbursts ever leads to collapse. Therefore, we turn to single compact objects for information about the angular momentum of stellar cores at the times of their collapses.

Pulsars are observed with spin periods between 0.03 and several seconds. If the Crab pulsar had been born with a period much faster than its present 0.03 s, the difference in rotational kinetic energy would be much larger than the observed radiated energy, the observed kinetic energy, or the inferred energy of relativistic particles. There is no direct evidence concerning the initial periods of other pulsars. For nonmagnetic DA white dwarfs, well observed objects have spin periods of at least 30 min (ref. 6). There is no evidence for any single nonmagnetic white dwarfs with shorter periods. ZZ Ceti stars may have periods of ~1 d (ref. 7). Magnetic white dwarfs are known[8] with periods of 2 hours and a day; others may have periods >100 yr. Single white dwarfs should not have changed their spin periods appreciably since their formation, unless the periods are many years, in which case interstellar accretion torques may be significant.

For a star flattened by rotation $Q \approx r^5\omega^2/G$, where ω is the spin frequency in rad s^{-1}. During collapse the specific angular momentum l is approximately conserved, and Q changes on the same time scale as the collapse. At neutron star densities where the radiation is produced, $t \approx 20GM/c^3$. Then, using crude conventional estimates as before

$$E \approx Pt \approx \frac{Gt}{5c^5}|\dddot{Q}|^2 \approx \frac{Gt}{5c^5}\left(\frac{rl^2}{Gt^3}\right)^2 \approx \frac{r^2l^4}{5c^5Gt^5} \tag{5}$$

For ordinary stellar cores $t \approx 10^{-4}$ s, $r \approx 10^6$ cm, and the efficiency is given by

$$\varepsilon \equiv \frac{E}{Mc^2} \approx \frac{r^2l^4}{5GMc^7t^5} \approx (10^{-68}\ \text{s}^4\ \text{cm}^{-8})l^4 \tag{6}$$

Table 1 gives the values of l and ε corresponding to various objects. The value for the neutron star rotating at breakup is within an order of magnitude of a detailed calculation[9], indicating the adequacy of our estimates. A white dwarf with a 71-s period would have $l \approx 3\times10^{16}$ cm^2 s^{-1}, exceeding l for a neutron star at breakup. Angular momentum would prevent the dynamic collapse of such an object to neutron star densities, reducing ε far below the value corresponding to the maximally rotating neutron star; ε is not a monotonic function of l. The collapse stops at a radius $r \approx l^2/GM$ on a time scale $t \approx (r^3/GM)^{1/2}$; equation (5) then leads to

$$\varepsilon \approx \frac{1}{5}\left(\frac{GM}{cl}\right)^7 \approx (5\times10^{108}\ \text{s}^{-7}\ \text{cm}^{14})l^{-7} \tag{7}$$

The further collapse of such an object is slow, because it depends on processes of angular momentum transport. Equation (7) is used on the fourth line of Table 1. Only for a narrow range of l can ε approach the value for a neutron star rotating at breakup.

The values of ε in Table 1 are again disappointingly small, especially when one recalls that both the neutron star rotating at breakup and the 2,000-s white dwarf are hypothetical objects, and that the 71-s white dwarf is the product of unusual circumstances.

It is possible that stellar collapse is an efficient ($\varepsilon \approx 10^{-3}$) source of gravitational radiation, but this is not implied by any existing data. If we try to set a semi-empirical lower limit we find $\varepsilon \approx 10^{-11}$ from the rotation of the Crab pulsar, or $\varepsilon \approx 10^{-10}$ from that of the white dwarf with a 2-h period. The recoil velocity of pulsars may suggest $\varepsilon \approx 10^{-9}$. As pointed out by Smarr[10] it is important to obtain further data on the rotation of white dwarfs and the initial spins of neutron stars.

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North Atlantic Deep Water formed by the later middle Miocene

P.-L. Blanc*, D. Rabussier†, C. Vergnaud-Grazzini† & J.-C. Duplessy*

* Centre des Faibles Radioactivités, Laboratoire mixte CNRS-CEA, 91190 Gif sur Yvette, France
† Département de Géologie Dynamique, Université Pierre et Marie Curie, 4 Place Jussieu, 75230 Paris Cédex 05, France

There is evidence that in Mesozoic and Palaeogene times both north and south subpolar areas had a mild climate[1,2]. Unlike the present day climatic symmetry, with warm equatorial regions and ice-covered polar regions, a strong planetary asymmetry developed in the middle Miocene, when the Antarctic ice cap was established while the northern high latitudes remained unglaciated[3]. The symmetry was restored when a Northern Hemisphere continental ice cover was established ~3 Myr ago in the middle Pliocene[4]. These changes influenced the formation of deep water in the ocean. At present the cold, dense bottom waters originate in only two high latitude areas—the Weddell Sea producing Antarctic Bottom Water, and the Norwegian Sea, producing Norwegian Sea Overflow Water which after mixing is a major component of the North Atlantic Deep Water. The production of cold deep water in the Southern Ocean started at the Eocene–Oligocene boundary[3]. We present here new evidence from isotope analyses of benthic Foraminifera from DSDP site 116 indicating that the production of oxygenated deep water in the North Atlantic Ocean started in the late middle-Miocene, ~12 Myr ago.

Site 116 (57°29′ N–15°55′ W) is on the Rockall Plateau, a shoal area at a depth of ~1,000 m. It is separated from Ireland and the Porcupine Bank by the Rockall trough and is on the present path of Norwegian Sea Overflow Water which spills through the Faeroe sill into the North Atlantic[5]. The Rockall Plateau has a continental basement and had already subsided to a depth of >1,000 m by the end of the Oligocene. The physiography of the area has not changed during the whole Neogene[5]. It is thus an ideal place to investigate the initiation of the production of cold deep water in the Northern Hemisphere.

Isotopic analyses have been performed on benthic Foraminifera from almost every section of cores 2–18, hole 116 and cores 8–11, hole 116A. Our analytical procedures[6] are identical to those described by Shackleton[7,8]. Results are expressed as

$$\delta = \left[\frac{R\text{ sample}}{R\text{ standard}} - 1\right] \times 10^3$$

where R is the isotopic ratio $^{18}O/^{16}O$ or $^{13}C/^{12}C$ and the standard used is the PDB standard.

In 71 samples, down to core 116–12, only aliquots composed entirely of *Cibicides* were analysed. In 22 samples, from core 116–15 to 18, mixed benthics have been used but the $\delta^{18}O$ results have been corrected by −0.23‰ and the $\delta^{13}C$ by +0.66‰ to make up for the mean difference observed in cores 116–11 and 12, where both *Cibicides* and mixed benthics have been analysed for calibration. Values will be tabulated elsewhere.

The $\delta^{18}O$ results are displayed in Fig. 1*a* together with those from sites 281 and 279, which are representative of the Southern Ocean, near the Antarctic Bottom Water source[3]. Only stratigraphic correlations[3,5] which are independent of the isotopic record have been used. These data indicate that the main $\delta^{18}O$ events (the late early-Miocene isotopically light peak, followed by an increase throughout the middle Miocene) are synchronous at these sites. We therefore consider that the stratigraphic correlations are satisfactory down to the late early-Miocene. The analyses at sites 279 and 281 were performed on *Uvigerina* or mixed benthics, while our site 116 analyses have been done on *Cibicides*, or corrected for departure from *Cibicides* when mixed benthics have been used. *Cibicides* has been shown[9,10] to be isotopically lighter than *Uvigerina* when taken from the same sediment. Moreover, site 279 is >2,000 m deeper than site 116 and is at present ~2 °C cooler. These effects combine to make the $\delta^{18}O$ values at site 116 consistently ~1% lighter than those for the southern sites. Nevertheless, data from both regions show trends similar to those already observed in other oceans[11–13]. We find generally low $\delta^{18}O$ values in the early Miocene sediments, up to core 116–10 (foraminiferal zones N8 and N9, ~16 Myr BP) followed by a general increase. This shift has been interpreted as corresponding to the establishment of the Antarctic ice cap[3,14]. This increase is not monotonic; oscillations of an amplitude of as much as 1‰ occur in $<10^5$ yr according to the published sedimentation rate of 2.4 cm kyr^{-1}. The values remain more stable, with a noise reduced to 0.5‰, during the late Miocene and early Pliocene (cores 116–6 to 2, 116A–11 and 10). A second much smaller increase in $\delta^{18}O$ is then registered, probably reflecting the inception of continental ice sheets in the Northern Hemisphere at ~3 Myr BP (core 116A–9). The oscillations above this level mark the Quaternary glaciations. The similarity of the $\delta^{18}O$ records at site 116 and in the other oceans during the Miocene and the early Pliocene time shows that the thermal history of the deep North Atlantic has been the same as that of the other deep water masses.

We also measured the $\delta^{13}C$ of benthic Foraminifera, which primarily reflects the $^{13}C/^{12}C$ ratio of the CO_2 dissolved in the deep water. $\delta^{13}C$ values will be modified if the rate of cycling through the exchangeable CO_2 reservoir changes. The two main causes of such a change are: (1) a modification of the volume of one part of the exchangeable CO_2 reservoir, for instance, an increase or a decrease in the continental biomass[9]. Such a modification, being extra-oceanic, must be felt equally in all oceans and the $\delta^{13}C$ must change everywhere in the same way; (2) a change in the residence time of the deep water masses: a longer residence time favours the conversion through oxidation of ^{13}C-depleted carbon from marine organic matter to dissolved bicarbonate. The longer the residence time, the lower the $\delta^{13}C$ (refs 15–17). This cannot explain a change in the $\delta^{13}C$ of the world ocean as a whole: the rate of cycling of carbon through the ocean can only be changed through a change in the turnover of the deep water masses. This in turn must have local causes, such as a change in the ratio of production of cold, dense bottom water, but such causes would have different effects on the $\delta^{13}C$ of the various ocean basins according to their distance and accessibility from the source.

Figure 1*b* compares the $\delta^{13}C$ for site 116 and for sites 279 and 281. Both curves begin with rather low values in the lowermost Miocene section. Towards the end of the early Miocene there is a fairly sharp increase in $\delta^{13}C$; this increase is of similar magnitude at both sites: 1.1‰ at site 279 and 1.2‰ at site 116. High values seem to persist longer in the Southern Ocean than in the North Atlantic, but this may be due to stratigraphic error in the comparison of the curves. During the middle Miocene the $\delta^{13}C$ values decrease to the previous early Miocene average at both sites.

So between the early and middle Miocene the parallelism in the trends of the $\delta^{13}C$ at both sites shows that the causes for the $\delta^{13}C$ changes were extra-oceanic. In contrast, we have no indication of a modification of the chemistry of CO_2 in the oceans, an indication that the residence time of the deep water masses remained constant.

After the late middle-Miocene (core 116–7, ~12 Myr BP) the carbon isotopic record in the North Atlantic acquires a character different from that of the South Pacific. While the $\delta^{13}C$ at site 281 continues to decrease, at site 116 there is a new increase in $\delta^{13}C$ of *Cibicides* of as much as 0.7‰ when measured on peak values. This is followed by stabilisation at values on average 0.3‰ above those of the earliest Miocene. These late Miocene $\delta^{13}C$ values are the same as those of Holocene *Cibicides* from

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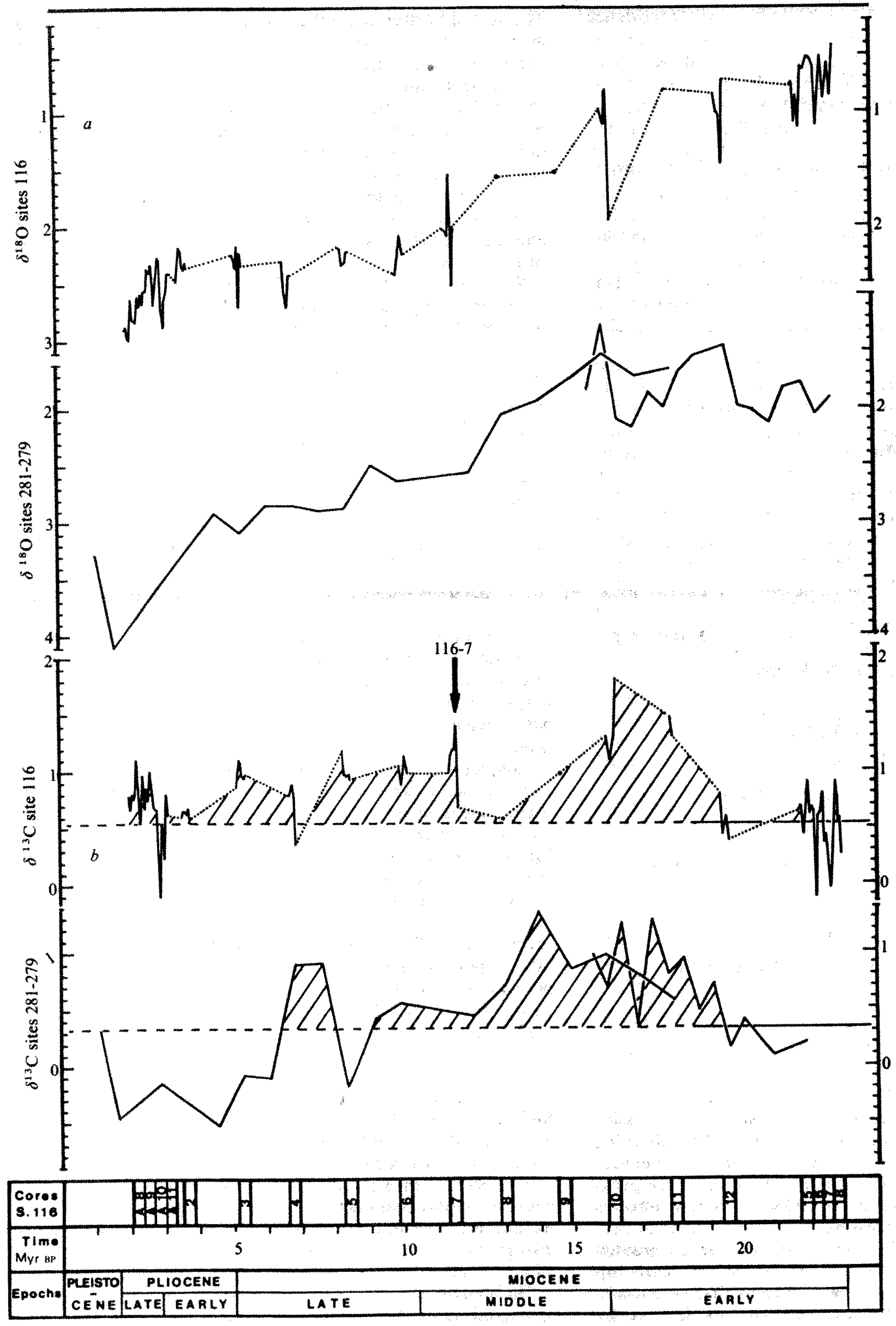

Fig. 1 Oxygen and carbon isotopic composition of benthic Foraminifera at DSDP site 116 (North Atlantic) compared to DSDP sites 279 and 281 (Southern Ocean). For comparison, a dashed line showing the average $\delta^{13}C$ for the early Miocene has been drawn throughout each ^{13}C curve and the area above this line has been shaded.

piston core CH 73139 C taken on the Feni ridge (J.-C.D., unpublished results). In the same way, at equatorial Atlantic site 366, the benthic Foraminifera, which also lived in the North Atlantic Deep Water, show $\delta^{13}C$ values similar to the present day values down to the late- to middle-Miocene boundary. Those values are ~0.8‰ below the late early-Miocene peak values, in comparison with 0.9‰ difference at site 116. The records are thus similar at both sites, but at site 366 a condensed sequence encompassing most of the middle Miocene precludes any accurate observations of the carbon shift[18].

Thus, at ~12 Myr BP the deep water in the North Atlantic Ocean became significantly richer in ^{13}C than before. No such enrichment is found in the Southern Ocean which indicates that it is a local North-Atlantic event. The present residence time of the North Atlantic Deep Water is short, and as the $\delta^{13}C$ of *Cibicides* has been of the same order during the past 12 Myr then this residence time must have been short during that period. Although the amplitude of the ^{13}C-shift in core 116–7 is difficult to estimate accurately, it is of the same order as the difference presently observed between the North and South

Pacific deep waters[15-17]. This shows that the previous residence time of the North Atlantic Deep Water was high, reflecting its great distance from the Deep Water source. No cold deep water was produced in this area as the coasts of the North Atlantic Ocean, which did not yet communicate with the Norwegian–Greenland sea, still enjoyed mild climates. The southern high latitudes were then the only sources of cold bottom water. The present influence of Antarctic Bottom Water is felt as far as 40° N in the Atlantic Ocean. Until the middle Miocene, it must have been felt even further north, as it now does in the North Pacific.

We have assumed that the late middle-Miocene age for the initiation of North Atlantic Deep Water depends on the stratigraphic interpretation of the fossil record at site 116. This interpretation was made difficult by the discontinuous coring, the severe drilling disturbance and the high latitude of the site. The date of 12 Myr BP for the same event is even more tentative, as it also relies on correlations between biostratigraphic events and radiometric dates.

Still, this date is in remarkable agreement with the age assumed for the subsidence of the main platform of the Iceland–Faeroe ridge below sea level[19,20]. The area of production of cold dense water must thus have been north of the ridge, either in the Norwegian Sea as it is today or further north in the Arctic Ocean. The appearance of this water south of the ridge is mainly a consequence of a tectonic process, and not of a climatic event, although cold winters are necessary to initiate the sinking of the surface water.

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Late Miocene palaeo-oceanography of the Atlantic: oxygen isotope data on planktonic and benthic Foraminifera

R. K. Matthews, W. B. Curry, K. C. Lohmann & M. A. Sommer

CENOP, Department of Geological Sciences, Brown University, Providence, Rhode Island 02912

R. Z. Poore

CENOP, US Geological Survey, Menlo Park, California 94025

The oxygen isotopic compositions of tests of Foraminifera are quantitatively related to global ice volume[1,2] and local temperature and salinity conditions in which the Foraminifera grew[3-8]. Thus, a well-preserved sample of deep-sea core contains information about sea-surface temperature (shallow-dwelling planktonic Foraminifera), structure of the thermocline (deeper-dwelling planktonic Foraminifera), and bottom water temperature (benthic Foraminifera), and the global ice volume effect is also superimposed. The progress which has been made in calibration of core–top isotopic data to modern oceanographic data[5,7,8], has led us to test state-of-the-art core–top methodology and calibration on a late Miocene time slice (6.0 ± 0.5 Myr BP) of the Atlantic Ocean. Samples studied can be related to either uppermost Zone NN11 or lowermost NN12 of Martini[9] and uppermost Zone N17 of Blow[10].

Our experimental design was as follows. We selected core samples representing a wide range of latitude because simultaneous consideration of bottom water conditions and high-latitude and low-latitude surface conditions imposes important constraints on interpretation of isotopic data. Then we sampled tightly controlled size fractions of numerous species of both planktonic and benthic Foraminifera from each sample. By sampling numerous species, our data should include the isotopically lightest and heaviest of the planktonic Foraminifera. We evaluated possible diagenetic alteration of Foraminifera wall structure using cathodoluminescence and scanning electron microscopy. Recrystallisation and/or precipitation of calcite after sediment deposition can seriously affect palaeo-oceanographic information. Finally, we compared analytical results with calculated values for the modern ocean and other explicit models.

Samples selected for study were washed, picked, and ultrasonically cleaned at the US Geological Survey, Menlo Park, taking care not to expose materials to high temperature or harsh chemicals. (Taxonomic identifications were done by R.Z.P.). Machine-ready Foraminifera samples were then shipped to Brown University where they were roasted *in vacuo* at 380 °C for ~1 h, reacted *in vacuo* with water-free polyphosphoric acid at 50 °C, and analysed on an on-line VG Micromass 602D mass spectrometer. PDB calibration via calcite standards from three other laboratories is within ±0.2‰ of these laboratories. Ten percent of all data were randomly duplicated with an average $\frac{1}{2}\Delta \pm 0.06$‰ for $\delta^{18}O$ and ±0.08‰ for $\delta^{13}C$. Our results are presented in Table 1 and Fig. 1. (Analytical details are available from the authors.)

Species lists in Table 1 are ranked on the basis of oxygen isotopic composition of the largest size fraction analysed, isotopically lightest (shallowest-dwelling planktonic Foraminifera) to isotopically heaviest (benthic Foraminifera closest to equilibrium). Note that although benthic $\delta^{18}O$ values in DSDP 116, 5/5, 119–121 cm are extremely light, we report these data because there is no objective reason to reject these analyses. As we did not have sufficient material to repeat the analyses we disregard all data from this sample in favour of data on 116, 5/5 143–145 cm.

The similarity between $\delta^{18}O$ species ranking in Table 1 and analogous core–top data is encouraging. High latitude sites (408 and 116) exhibit <1‰ variation among planktonic species whereas low latitude sites (98 and 366A) exhibit approximately 3‰ variation from isotopically lightest to isotopically heaviest planktonic Foraminifera. These relationships are quite similar to core–top studies[5,8]. Further, *G. sacculifera, G. triloba, O. universa, G. cultrata* (s. 1.), and *G. scitula* are all well known from plankton tow and core–top[5,7,11,12,13] and display generally similar rankings in most of our late Miocene samples.

Figure 1 compares late Miocene and modern $\delta^{18}O$ values with latitude. Late Miocene palaeo-oceanographic conditions are deduced by comparing the Miocene data with the appropriate values calculated from modern conditions[8].

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Table 1 Stable isotopic data for size fractions of various species of planktonic and benthic Foraminifera in a 6.0 ± 0.5 Myr BP transect of the Atlantic from Iceland to Rio Grande Rise

Sample	$\sigma^{18}O$ (‰, PDB)	$\delta^{13}C$ (‰, PDB)
DSDP Site 408, 63°23′ N, 28°55′ W, 1,624 m		
Core 15/5, 30–32 cm		
Globigerina bulloides, 412 μm±	+1.69	−0.01
Globigerina bulloides, 275 μm±	+2.59	−0.88
Neogloboquadrina atlantica, 500 μm±	+2.30	+1.15
Neogloboquadrina atlantica, 425 μm±, (2)	+2.20	+0.78
Globocassidulina subglobosa (benthic), 350–450 μm, (2)	+3.03	+0.50
DSDP Site 116, 57°30′ N, 15°56′ W, 1,161 m		
Core 5/5, 119–121 cm*		
Globigerinita sp. 375 μm±	+1.53	+0.67
Globigerina eamesi, 375 μm±	+2.00	−0.43
Neogloboquadrina atlantica, 375 μm±	+2.07	+0.63
Neogloboquadrina atlantica, 300 μm±	+1.20	+0.81
Globigerina bulloides, 500 μm±	+2.82	+0.13
Globigerina bulloides, 375 μm±	+1.62	+0.02
Globigerina bulloides, 250 μm±	+3.06	−0.07
Cibicidoides kullenbergi (s. 1.) (benthic), 250–425 μm	+1.07	+0.44
Planulina wuellerstorfi (benthic), 400–750 μm	+1.17	+0.71
Core 5/5, 143–145 cm		
Neogloboquadrina atlantica, 375 μm±, (2)	+0.96	+0.72
Globigerina bulloides, 300–355 μm	+1.56	+0.25
Globigerina bulloides, 212–250 μm	+0.97	−0.09
Globorotalia conomiozea (s. 1.), 475 μm±	+1.59	+0.77
Globorotalia conomiozea 300 μm±	+1.35	+0.43
Globigerina eamesi, 300–355 μm	+1.75	−0.28
Globigerina eamesi, 212–250 μm	+1.28	−0.70
Globocassidulina subglobosa (benthic), 180–250 μm	+2.55	+0.26
Globocassidulina subglobosa (benthic) <180 μm	+2.45	−0.11
DSDP Site 335, 37°17′ N, 35°12′ W, 3,198 m		
Core 4/3, 79–81 cm		
Globigerinoides sacculifera, 300–600 μm	−0.60	+0.76
Globigerinoides triloba, 300–600 μm	−0.04	+1.82
Globigerina nepenthes, 400 μm±	+0.55	+0.83
Globigerina nepenthes, 200–300 μm±	+0.59	+0.63
Globorotalia scitula, 300–400 μm	+0.66	−0.85
Globorotalia scitula, 250 μm±	−0.31	−0.75
Orbulina universa, 600 μm±	+1.28	+1.21
Orbulina universa, 300 μm±, (2)	+0.61	+1.08
Planulina wuellerstorfi (benthic), 750–900 μm	+2.49	+0.67
Globocassidulina subglobosa (benthic), 450–700 μm	+3.34	−0.03
Globocassidulina subglobosa (benthic), 375–450 μm	+3.54	+0.03
DSDP Site 98, 25°32′ N, 77°19′ W, 2,769 m		
Core 3/3, 14–16 cm		
Hastergerina siphonifera, >500 μm	−1.48	−0.34
Hastergerina siphonifera, <250 μm	+0.67	−0.41
Orbulina universa, >500 μm±	−1.03	+1.72
Orbulina universa, 375 μm±	−0.20	+1.59
Orbulina universa, <250 μm±	+0.42	+0.93
Globigerinoides sacculifera, 550 μm±	−0.79	+2.01
Globigerinoides triloba, >500 μm±, (2)	−0.68	+2.26
Globigerinoides triloba, 375 μm±	−0.31	+0.95
Globigerinoides triloba, 300 μm±	−0.29	+0.90
Globigerinoides triloba, <250 μm	+0.24	+0.61
Globigerinoides obliqua, >375 μm	−0.16	+0.92
Globigerinoides obliqua, <250 μm	+0.10	+0.62
Globorotalia cultrata (s. 1.), 500 μm±, (2)	−0.01	+0.38
Globorotalia cultrata, (s. 1.), 400 μm±	−0.11	+0.46
Globorotalia scitula, >350 μm	+1.69	+0.24
Globigerina nepenthes, >375 μm	+1.90	+0.74
Globigerina nepenthes, <250 μm	+0.47	−0.33
DSDP Site 98, 25°23′ N, 77°19′ W, 2,769 m		
Core 3/3, 17–21 cm		
Neogloboquadrina acostaensis, 312 μm±	−0.17	−.1.32
Neogloboquadrina acostaensis, 250 μm±	+0.24	−1.88
Globorotalia scitula, 437 μm±	+1.75	+0.05
Globorotalia scitula, 350 μm±	+1.07	−0.11
Globorotalia scitula, 287 μm±	+1.10	−0.06
Globocassidulina subglobosa (benthic), 540 μm±	+3.18	+0.15
DSDP Site 366A, 5°41′ N, 19°51′ W, 2,860 m		
Core 12/1, 99–101 cm		
Globigerinoides sacculifera, 500–800 μm	−1.20	+2.76
Globigerinoides sacculifera, 200–300 μm, (2)	−1.13	+1.36
Globorotalia cultrata (s. 1.), 600–800 μm	−0.19	+1.59
Globoratalia cultrata (s. 1.), 400 μm±	+0.46	+1.08
Globoquadrina altispira, 600–700 μm	−0.08	+1.96
Globoquadrina altispira, 500 μm±	−0.34	+2.16
Orbulina universa, 800–1,000 μm	+0.59	+1.99
Orbulina universa, 600 μm±	+0.55	+2.06
Globorotalia scitula, 800–1,000 μm	+1.29	+0.73
Globorotalia scitula, 400 μm±, (2)	+1.23	+0.49
Planulina wuellerstorfi (benthic), 380–750 μm	+2.43	+0.36
Globocassidulina subglobosa (benthic), 275–640 μm	+2.57	−0.56
CH115–86, 30°S, 35°34′ W, 2,122 m		
554 cm		
Globigerinoides sacculifera, >500 μm, (4)	+0.52	+2.60
Globigerinoides sacculifera, 425 μm±, (2)	+0.74	+2.20
Globigerinoides sacculifera, 287 μm±	+0.55	+1.95
Globorotalia conomiozea (plexus), 520 μm± (encrusted)	+1.01	+2.26
Globorotalia conomiozea (plexus), 400 μm± (not encrusted)	+1.43	+1.55
Globorotalia conomiozea (plexus), 300 μm± (not encrusted)	+1.58	+1.42
Planulina wuellerstorfi (benthic), 525–825 μm	+2.75	+1.53
702 cm†		
Globigerina nepethes, >500 μm	+0.64	+1.20
Globigerina nepenthes, 375 μm±	+0.55	+1.24
Globigerina nepenthes, 250–355 μm	+0.85	+1.77
Globigerina nepenthes, <250 μm	+0.36	+0.28
Globigerinoides triloba, >500 μm	+0.94	+2.12
Globigerinoides triloba, 355–500 μm	+1.01	+2.42
Globigerinoides triloba, 250–355 μm	+1.48	+1.79
Globigerinoides triloba, <250 μm, (2)	+2.12	+1.69
Orbulina universa, >500 μm, (2)	+1.34	+2.25
Orbulina universa, 355–500 μm, (2)	+1.38	+2.25
Orbulina universa, 250–355 μm	+1.11	+1.89
Globoquadrina dehiscens, >500 μm	+1.57	+1.68
Globoquadrina dehiscens, 355–500 μm	+1.45	+1.91
Globoquadrina dehiscens, <250 μm	+3.17	+0.91
Globorotalia conomiozea (plexus), >500 μm	+2.27	+1.69
Globorotalia conomiozea (plexus), <250 μm	+2.85	+1.04
Planulina wuellerstorfi (benthic), 500–800 μm	+2.64	+1.18
Globocassidulina subglobosa (benthic), 300–550 μm	+2.99	+0.38

Planktonic species lists are ranked on the basis of $\delta^{18}O$ of the largest size fraction.

* We disregard data on this sample in favour of data on 116, 5/5, 143–145 cm.

† We disregard data on this sample in favour of data on 554 cm; diagenesis apparent.

Four features of Fig. 1 are important for interpreting our late Miocene data. First, late Miocene $\delta^{18}O$ benthic data values for *P. wuellerstorfi* and *G. subglobosa* differ from calculated modern benthic equilibrium values by −1.0‰ and −0.5‰, respectively. These values match observed Holocene departures of these species from equilibrium. Second, the isotopically lightest late Miocene values at each of the three North Atlantic sites (408, 116, and 335) are in remarkable agreement with calculated values for the modern summer. A similar correlation of isotopic data to summer conditions has been noted in core-top studies[8]. Third, consider the relation of isotopically heaviest planktonic Foraminifera species to the value calculated for the modern equal density surface, $\sigma_T = 26.8$. Curry has shown this surface to be the habitat of deepest-dwelling *Globorotalia* in core-top and 18,000 yr BP materials from the Indian Ocean[8,14]. Note the outstanding agreement in Fig. 1 between isotopically heaviest late Miocene planktonic Foraminifera and the calculated $\delta^{18}O$ values for the modern $\sigma_T = 26.8$ surface. Finally, there is a general discrepancy between isotopically lightest values at Sites 98, 366A, and CH115-86 and the calculated values for modern

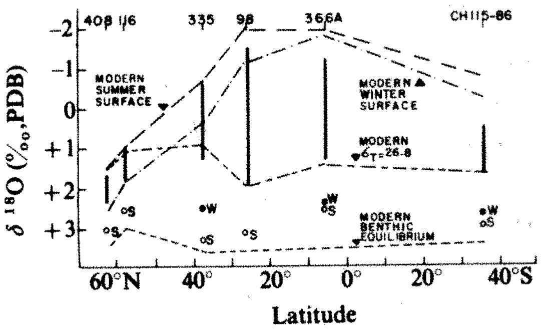

Fig. 1 Transect of the Atlantic Ocean depicting late Miocene observed and modern expected δ^{18}O values on planktonic and benthic Foraminifera plotted versus latitude. Numbers across the top are sample sites. Late Miocene δ^{18}O data on planktonic Foraminifera (bars) and benthic Foraminifera (○, *G. subglobosa*; ●, *P. wuellerstorfi*). Dashed lines represent modern expected δ^{18}O values for equilibrium with summer and winter sea-surface conditions, the 26.8 σ_T surface and bottom water. Observed late Miocene data agree well with modern expected values except for anomalously heavy surface isotopic values for Sites 98, 366A, and CH115-86. The discrepancy at CH115-86 is beyond the error of measurements and calculations and, therefore, requires explanation. See text for the three alternative hypotheses.

surface conditions. In particular the discrepancy between the data for CH115-86 and the calculated values demands an explanation.

Although three of these comparisons suggest the late Miocene Atlantic was indistinguishable from the modern, the final one contradicts this conclusion. To bring these comparisons into agreement over similarities and differences between late Miocene Atlantic and the modern, we consider three hypotheses: (1) late Miocene ice volume was larger than today; (2) site CH115-86 was significantly colder in late Miocene than it is today; and (3) the surface-dwelling Foraminifera at CH115-86 yield heavy δ^{18}O values because they have undergone significant shallow burial diagenesis.

(1) If the discrepancy between late Miocene and modern at CH115-86 is attributed to greater ice volume in late Miocene, then this effect must also be superimposed on sea-surface and benthic data for all other sites[1,2]. The agreement between benthic data and high latitude North Atlantic surface data, and modern conditions, means that any proposal for an increased ice volume must be accompanied by a proposal of increased water temperature in those sectors. While such a proposal is not out of the question, we suggest that global ice volume, Atlantic bottom water temperatures, and North Atlantic sea surface temperatures in late Miocene were similar to modern and seek some other explanation for the discrepancy at CH115-86.

(2) If one considers that site CH115-86 really was four or five degrees colder in late Miocene than it is today, one plausible palaeo-oceanographic cause of this cooling is that surface gyre circulation was less intense in the late Miocene. This would reduce the zonal anomaly at Sites 98 and CH115-86, making both sites cooler than today. However, faunal evidence that argues against this interpretation. Planktonic Foraminifera assemblages (>149 μm size fraction) in modern sediments from the vicinity of CH115-86 contain ~5% of the tropical Foraminifera, *G. sacculifera*[15]. Counts of the >177 μm size fraction of CH115-86 samples show that *G. sacculifera* is at least 10% and sometimes as much as 20% of late Miocene faunal assemblages. Therefore, we assume sea surface temperatures in the vicinity of CH115-86 were not significantly cooler during the late Miocene than they are today.

(3) There is good reason to believe that the samples at CH115-86 (and those at Site 98) have undergone some shallow burial diagenesis. Both sites are situated above the modern aragonite compensation depth. If aragonite is buried with the sediment, the greater solubility of aragonite surely drives a dissolution/reprecipitation process through which calcite is precipitated onto pre-existing calcite nuclei such as planktonic Foraminifera. In the simplest situation, this new calcite grows from pore fluid with oxygen isotopic composition similar to bottom water and at bottom water temperatures. Precipitation of such calcite would bring isotopic values for surface-dwelling planktonic Foraminifera closer to those observed for benthic Foraminifera. The isotopic data for sample CH115-86, 702 cm, is consistent with this hypothesis. Isotopic values for benthic Foraminifera in this sample are consistent with values obtained on CH115-86, 554 cm, yet all values of *G. triloba* in the 702 cm are significantly heavier than the values for *G. sacculifera* in the 554 cm sample, and the fine fraction *G. triloba* and *G. dehiscens* in the 702 cm sample have isotopic values approaching those of benthic Foraminifera. Examination of the *G. sacculifera* material from the CH115-86 samples gives a clear indication of precipitation of calcite cement onto the interior wall of the foraminiferal tests as well as partial dissolution of original wall material.

Three out of four important comparisons between modern Atlantic and late Miocene Atlantic, yield indistinguishable δ^{18}O values for the two oceans. We are optimistic that stable isotope data can be used to quantify several important aspects of palaeo-oceans and to place extinct species within their three-dimensional ecological context in still older palaeo-oceans. We are convinced that diagenesis can seriously compromise the palaeo-oceanographic integrity of foraminiferal isotopic data, but that it can be recognised and avoided. Care should be taken in future studies on samples which may have been deposited above the aragonite compensation depth, and scanning electron microscopy and chemical studies should be used to demonstrate the palaeo-oceanographic integrity of materials submitted to isotopic analysis.

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Isotopic data for scleractinian corals explain their palaeotemperature uncertainties

Peter Koenraad Swart*

Department of Geology, King's College, University of London, London WC2R 2LS, UK

Max Laurence Coleman

Institute of Geological Sciences, 64–78 Gray's Inn Road, London WC1X 8NG, UK

New data on the variability of the oxygen isotope composition of water on a coral reef reveal that there are large changes in δ^{18}O linked to high evaporation rates and precipitation. This together with variation in growth rate is sufficient to mask large water temperature variations, which otherwise would be reflected in

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* Present address: Department of Mineralogy and Petrology, University of Cambridge, Downing Place, Cambridge, UK.

the skeletal oxygen isotope ratio. The carbon isotope ratio in the skeleton, however, does vary probably as a result of growth rate. In contrast to previous studies only the most recently formed part of the skeleton was used to reduce the possibility of diagenetic additions which would distort the results.

Calcium carbonate precipitated from water by marine organisms has an oxygen isotope composition which is temperature dependent[1,2]. This has been used for Foraminifera which secrete carbonate in equilibrium with seawater to map palaeotemperature variations through the Pleistocene[3]. Weber and Woodhead[4] have shown that although corals fractionate both the carbon and oxygen isotopes, the slope of the temperature relationship is similar to that determined by Epstein *et al.*[2]. Most corals are characterised by alternating bands of dense and less dense skeleton. The denser layer is believed to be a result of thickening of the skeletal components and a closer bundling of the aragonite crystals. Attempts to use the temperature dependence of the ^{18}O ratio to determine in which part of the year the denser bands were formed have led to conflicting conclusions[5–7]. From isotopic evidence Weber *et al.*[5] concluded that the bands were formed during the warmer part of the year and although similar trends were noticed by Goreau[6] he discounted the trend as the corals which he examined formed their dense bands in the winter when the temperature is low. Emiliani *et al.*[7] suggested that the denser bands could be produced in both summer and winter, however, they also noted a broad inverse correlation between $\delta^{13}C$ and $\delta^{18}O$ in contrast to other researchers who found positive correlations[8–10] and Goreau who found no correlation at all[6]. In contrast to oxygen, the carbon isotope ratio may be related to growth rate. Emiliani *et al.*[7] concluded that during the slower growth of the coral less fractionation occurred and therefore $\delta^{13}C$ approached that of seawater. However, considering other reports[8,11] that corals growing at depth are depleted in ^{13}C with respect to corals growing near the surface, this explanation apparently is not plausible, as surface corals generally have much higher growth rates[12].

The palaeotemperature equation developed by Epstein *et al.*[2] and modified by Craig[13]

$$T = 16.9 - 4.21(\delta c - \delta w) + 0.14(\delta c - \delta w)^2$$

shows that the oxygen isotope composition of a carbonate is not only a function of temperature but also depends on the isotope composition of the water; $\delta c - \delta w$ is the measured difference between the carbonate and water. For seawater, variations in $\delta^{18}O$ are related to changes in salinity[14], but previously, local and short-term variations were considered to have a negligible effect on the $\delta^{18}O$ of skeletal carbonates. In an attempt to examine these effects on the aragonite skeletons of a scleractinian reef coral, samples of water and specimens of *Acropora cuneata* were collected in January 1978 from three sites across the reef profile at Heron Island, Australia.

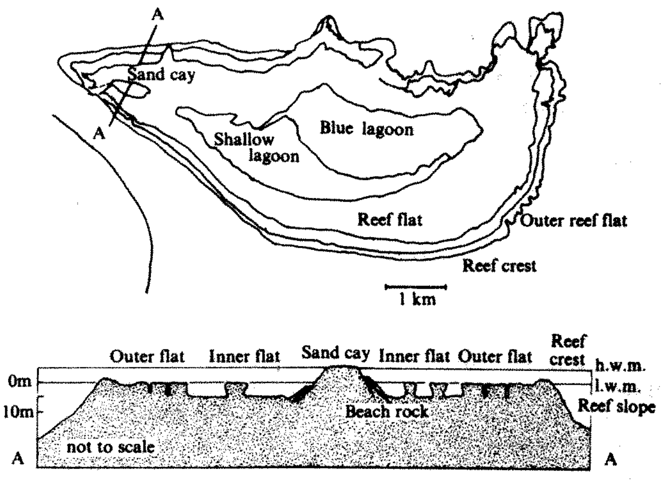

Fig. 1 The physiographic zonation and cross-section of Heron Island Reef.

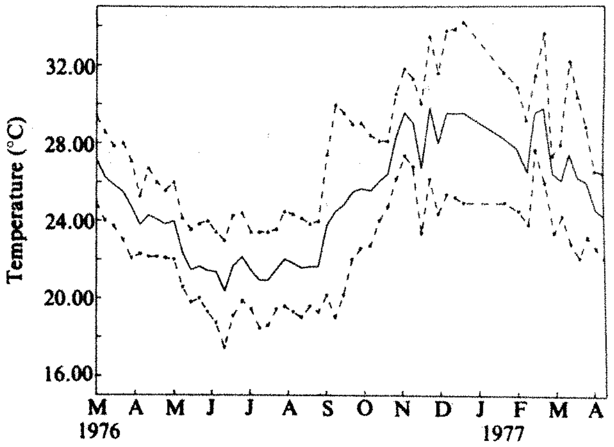

Fig. 2 Average weekly seawater temperatures on the reef flat at Heron Island, March 1976–April 1977 (unpublished data from D. Potts). The maximum and minimum temperatures are shown by the dashed lines.

Heron Island is a typical lagoonal platform reef[15], near the southern end of the Great Barrier Reef, showing standard reef zonation (see Fig. 1). The reef flat has been shown to experience diurnal temperature variations as high as 12 °C (ref. 16) (Fig. 2), whereas off the reef temperature ranges are small. These variations arise at low tide, during which there may be several hours of slack water. At this time, the semi-isolated pools on the reef flat may become subjected to intense heating resulting in changes in temperature and salinity. However, changes in salinity also result from evaporation caused through high wind speeds and dilution due to rainfall. Water samples were collected over a 3-month period, mainly from the reef flat, to determine the range of salinity and $\delta^{18}O$ variation. These results are shown together with the data of Lowenstam and Epstein[14] in Fig. 3.

For the carbonate isotope analysis only the terminal growth portion of each specimen was used to avoid the possibility of any diagenetic carbonate being present. Diagenetic additions occur very shortly after initial growth and their presence would distort the result. Before analysis, the organic material known to be intimately associated with the skeleton must be removed, as the organic molecules in the extracted gas interfere with the analysis: (the effects of the organic material has been summarised elsewhere[11]). As removal of organic material with sodium hypochlorite solution or roasting in a helium atmosphere may alter the isotope ratio, the organic material was removed in a low temperature oxygen plasma furnace. Periods of up to 12 h had no affect on the isotopic composition of either control samples or the laboratory standard.

For isotopic analysis, carbon dioxide was extracted from the carbonate by the normal method, reaction with phosphoric acid[17]. This process involves an isotopic fractionation of oxygen which is different for calcite and aragonite (1.01025 and 1.01034, respectively[18]). As the samples were analysed with reference to a calcite standard, the $\delta^{18}O$ values shown in Fig. 4 were corrected for this effect (as well as the usual isobaric interferences and instrumental effects[19,20]). Similarly, calcite and aragonite precipitated at the same temperature have different isotopic compositions[21,22] for which a correction must be made if attempting to calculate temperature, or isotopic composition of the water. Previous papers on corals[5–11] have not stated whether any of these correction factors were applied.

Corals growing on the reef flat (Fig. 1) might be expected to reflect the large temperature variations as well as the changes in the $\delta^{18}O$ content of the water in their skeletons. Corals growing on the reef slope which is a much more stable environment should be isotopically more uniform. However, Fig. 4 shows that there are no discernable differences in the $\delta^{18}O$ between the three environments even though the heaviest oxygen values occur off the reef flat as one might expect if there were a temperature relationship. As far as the $\delta^{13}C$ value is concerned, there is a statistically lower value on the reef slope, thereby confirming previous results[8,11]. Woodhead's[12] statement that corals at Heron Island on the reef slope have a much lower growth rate than those on the reef flat supports the idea of Goreau[6] that faster growing corals utilise more inorganic carbon relative to metabolic carbon which has more negative values.

If the present results are compared with similar data collected from the Heron Island locality by Weber and Woodhead[8], the values for the oxygen isotope ratios are 1‰ lighter. This apparent discrepancy may be a result of preparation procedure, as Weber and Woodhead[8] removed the organic material from their corals using the helium treatment; however, this should have made their corals lighter than ours, they are in fact heavier. Either there has been a drastic change in the prevalent environmental conditions or there are differences in the analytical techniques. Another possible source of contamination is subsequent diagenesis, either through isotopic exchange or the activities of endoliths such as fungi, algae and bacteria: boring filaments are known to infest coral skeletons within hours of it being formed[23]. Clearly it is practically impossible to eliminate all subsequent diagenetic affects. Sampling the growth bands within coral heads therefore will include the products of a wide range of diagenetic processes in the sampled carbonate; this is particularly relevant to the denser bands, which coincide with green algal bands often seen when a coral head is broken open[24].

Isotopic data obtained from all marine organisms must be interpreted with extreme caution. There are too many uncertainties concerning the controlling processes in modern calcareous organisms, without considering subsequent diagenetic effects. The reasons for the lack of differences in the skeletal $\delta^{18}O$ value between the various habitats in the present research, despite the large temperature differences, is probably a result of either or both of the following factors: (1) The variation in the seawater $\delta^{18}O$ alone would be sufficient to mask an actual temperature difference of 7 °C; however, as the $\delta^{18}O$ of seawater may vary sympathetically with temperature, an even greater temperature difference may be masked. (2) An increase in temperature increases the metabolic processes of the coral and therefore alters the proportions of oxygen and carbon incorporated from the various organic and inorganic sources proposed by Weber and Woodhead[8] and Goreau[6]. According to Goreau[6] an increase in growth rate increases the isotopically heavier inorganic component incorporated. This effectively cancels out the temperature dependence of the $\delta^{18}O$ value. As the $\delta^{13}C$ is only slightly temperature dependent, this change in growth rate is reflected in the carbon isotope composition.

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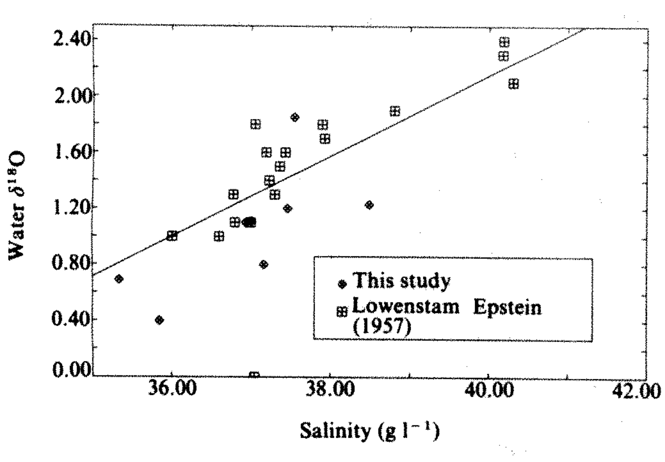

Fig. 3 Seawater $\delta^{18}O$ from Lowenstam and Epstein (1957) together with data from this study plotted against salinity. The regression line is for the data of Lowenstam and Epstein[14].

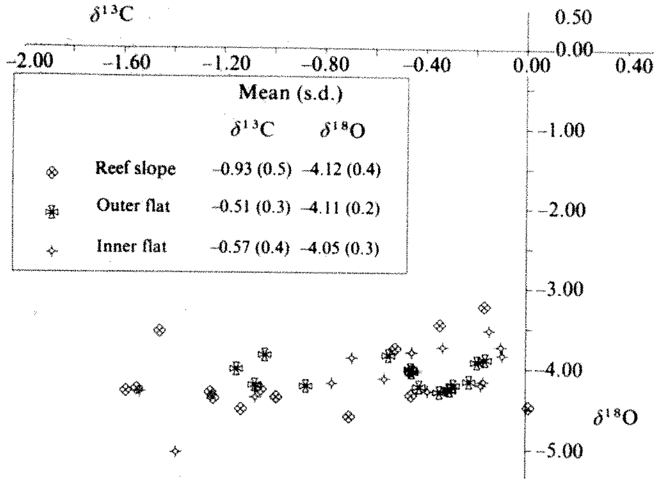

Fig. 4 Data for 45 specimens of *Acropora cuneata* collected from the inner flat, outer flat and reef slope. Values are corrected for the fractionation difference caused by dissolution in phosphoric acid, relative to calcite.

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Thermal effects of basalt on continental crust and crustal contamination of magmas

P. J. Patchett*

Department of Geology and Mineralogy,
Parks Road, Oxford, UK

A simple conduction-based heat-flow model is used to calculate the thickness of zones of continental crustal rock adjacent to basalt sheets which are raised to the temperatures of (1) hydrous mineral breakdown and (2) partial melting. Basalt emplacement leads to wide zones of partial fusion and aqueous fluid circulation at most crustal levels. Contamination of the basalt magma by partial melts or aqueous fluids is most effective where magmatism is episodic and repetitive on a 0.1–1,000 yr time scale.

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* Present address: US Geological Survey, Denver Federal Center, Denver, Colorado 80225.

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Table 1 Thermal properties and symbols

Property	Symbol	Value assumed
Distance	x	
Time	t	
Temperature	T	
Crustal rocks		
Density	ρ	$2.7\ \mathrm{g\ cm^{-3}}$
Heat capacity	C	$840\ \mathrm{J\ kg^{-1}\ K^{-1}}$
Thermal conductivity	K	$2.5\ \mathrm{W\ m^{-1}\ K^{-1}}$
Initial thermal gradient		$15\ \mathrm{K\ km^{-1}}$ (Fig. 1)
*Basalt magma**		
Latent heat of fusion†	L	$420\ \mathrm{kJ\ kg^{-1}}$
Liquidus T	T_{liq}	1200 °C (1,473 K)
Solidus T	T_{sol}	900 °C (1,173 K)
Volume fraction crystallised‡	V	

* Basalt was taken to have the same density, heat capacity and thermal conductivity as the crustal rocks; this has very little effect on the results.

† Crystallisation was assumed linear with temperature, and latent heat assumed to be liberated uniformly between liquidus and solidus temperatures.

‡ As a result of the above assumption derivative $\partial V/\partial t$ is obtained by the substitution $\partial V/\partial t = \partial T/\partial t \cdot 1/(T_{\mathrm{liq}} - T_{\mathrm{sol}})$ (see ref. 1).

The thermal calculations were performed using a program written by Wells[1] to solve by an implicit Crank–Nicolson finite difference method the one-dimensional equation

$$\frac{\partial T}{\partial t} = \frac{K}{\rho C} \cdot \frac{\partial^2 T}{\partial x^2} + \frac{L}{C} \cdot \frac{\partial V}{\partial t}$$

Symbols and assumed values for parameters are given in Table 1. This simplification of the full equation given in ref. 1 arises because radiogenic heat production is ignored, and the thermal conductivity treated as constant. The principles, and results, do not differ materially from those given by Jaeger[2], except that initial crustal temperature and its effect on the rate of heat transfer are taken into account.

Basalt sheets in any orientation are emplaced into the crust as a single pulse of magma. The heat flow equation is solved at right angles to these infinitely extensive sheets. Two thermal effects are considered:

(A) Aqueous fluid circulation in the crustal rock. Where a free fluid phase is present, it will move in response to the thermal gradient. Where there is no initial free fluid phase, H_2O movement will begin when hydrous minerals break down. The stability limits for common hydrous minerals are shown in Fig. 1. The zones of country rock raised above the breakdown temperatures of muscovite and biotite in the absence of quartz are shown in Fig. 2.

(B) Partial melting of the crustal rock. Almost all crustal rocks containing a free fluid phase can yield some partial melt at the H_2O-saturated granite minimum (Fig. 1)[3]. The zone of country rock heated above this curve is shown in Fig. 2. Where there is no free fluid phase, melting begins at hydrous mineral breakdown[3]. Therefore the probable zone of partial melting, shaded in Fig. 2, is bounded by the granite minimum to ~4 km, and by the breakdown of biotite below that depth. Movement of aqueous fluids and production of felsic partial melts must be real effects physically distinct from each other because there is a wide solubility gap between H_2O and silicate melt, spanning 70% of the compositional range even at 30 km depth[4].

The thermal model used may yield minimum estimates for the zones of crust affected by partial melting and fluid movement because:

(1) Flow of magma through the fissure will increase the overall heat input to the country rock.

(2) The calculations are based only on heat conduction, and heat transfer by convection in the magma and by circulating fluids in the crustal rocks will speed up the heat flow.

(3) At least in the upper crust, there is likely to be a free fluid phase. This will extend the zone of partial melting, and will be mobilised over a much greater width than the zone of hydrous mineral breakdown.

On the other hand, the estimates may be maxima because:

(4) If magmas rise through pipes rather than fissures, initial effects on crustal rocks will be unchanged, but the heated zones will later be narrower and shorter-lived.

(5) The circulation of fluids may have the effect of dissipating the heat quite rapidly, so that there is a very wide zone affected by fluids, but only a narrow partially fused zone.

(6) Many lower crustal regions may consist of granulite facies rocks, containing neither a free fluid phase nor significant hydrous minerals. Such rocks may not melt until 1,000 °C, and both melting and fluid phase circulation would be essentially absent. The dotted lines below 20 km in Fig. 2 reflect this uncertainty, but it is significant that high electrical conductivity in the lower crust of two areas, both in high-grade metamorphic regions, necessitate the presence of considerable pore water[5,6].

(7) Absorption of latent heat of fusion by the crustal rock is not taken into account, as it is difficult to estimate the degree of partial melting. Partial fusion of 50% over a zone results in a ~30% reduction of zone width as compared to Fig. 2, while 10% partial fusion leads to only ~8% width reduction. The effects are clearly marginal to the general model discussed here.

(8) If the hydrous phase in the crustal rocks is hornblende rather than sheet silicates, the breakdown zone will be narrower than that shown in Fig. 2, and in some cases absent.

Combining all these points suggests that the magnitude of the effects shown in Fig. 2 is probably realistic. The greatest uncertainty is in the lower crust.

Figure 2 emphasises the fact that in the uppermost crust, aqueous fluid circulation is the dominant effect of magma emplacement. At this level, partial melting does not occur due to the high temperature of granite minimum melting (Fig. 1). As hydrous mineral breakdown temperatures are very conservative indicators of the beginning of fluid circulation, the actual width affected by fluids in the upper 10 km will probably be at least double that shown. Norton and Taylor[20] have modelled such high-level effects in detail.

Figure 2 also demonstrates clearly that the basalt causes considerable partial melting of the crustal rocks at most depths. After an appropriate lapse of time, basalt sheets at 10 km depth for example, can develop partially molten zones of country rock equal to 10–20% of their own thickness. Less than 1 yr is

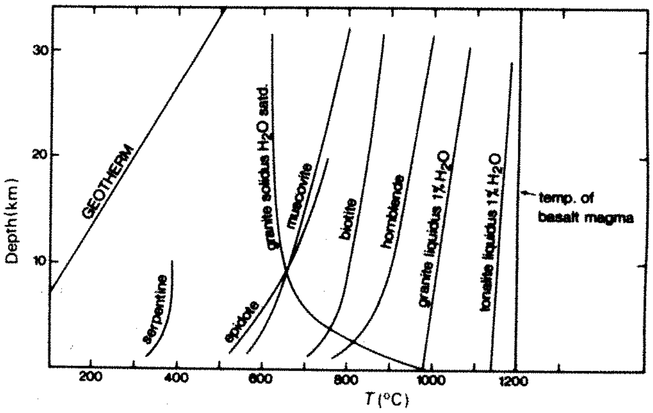

Fig. 1 The *P–T* equilibria relevant to emplacement of basalt magma into continental crust. The initial geotherm used is shown. Melting curves are from Wyllie[3]; serpentine breakdown from Johannes[16]; epidote breakdown under the haematite–magnetite buffer from Liou[17]; muscovite in the absence of quartz from Chatterjee and Johannes[18]; biotite (15% annite molecule, haematite-magnetite, quartz absent) from Wones and Eugster[19]; approximate hornblende curve from Wyllie[3]. Quartz absent reactions are used because there is probably insufficient time for minerals to interact; any other assumption leads to breakdown at lower temperature.

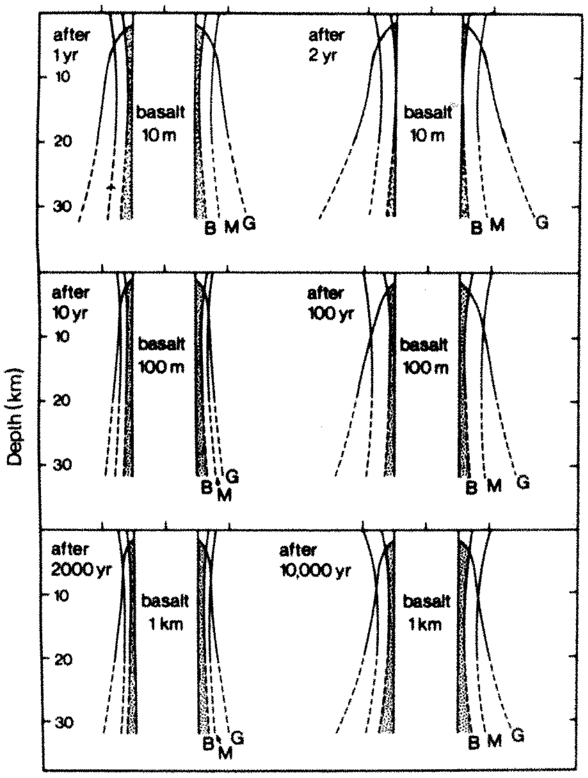

Fig. 2 The approximate temperature zones above biotite breakdown (limited by line B), muscovite breakdown (M) and the Ab–Or–Qz–V minimum melting (G) for basalt sheets 10, 100 and 1,000 m thick at 0–30 km depth. Dotting of lines below 20 km reflects uncertain H_2O and hydrous mineral content of lower crust. Shaded: the probable minimum limit of partial melting adjacent to the basalt.

required for this to be achieved around a 10 m basalt sheet, 10 yr for a 100 m sheet, and as much as 2,000 yr for a 1,000 m sheet. The upper example in Fig. 2 shows that any basalt sheet $\geqslant$10 m thick at 10 km depth develops partially molten zones $\geqslant$1 m on either side after <1 yr; clearly, some contamination of a magma by partial melts (or aqueous fluids) could take place if it flowed through its conduit for only a few weeks.

Total melting of the crust is possible only over a very short distance adjacent to the basalt, if at all. This is because while the first partial melts may be H_2O-saturated, the overall H_2O content of the crustal rock is unlikely to be >1% (ref. 4), so that its liquidus temperature will be >1,000 °C (Fig. 1)[3]. Melts can be produced over a wide zone, but they are always partial melts.

The present study emphasises the expected widespread nature of partial melting and aqueous fluid circulation near to mafic magma conduits. These selective mechanisms (see also refs 7–9 among many others) are inherently more probable for contamination of mafic magmas than bulk assimilation (compare, for example, ref. 10). Some studies[8,11–13] have shown that crystal–liquid or crystal–aqueous fluid isotopic disequilibrium occurs in the crust over the time scale required for contamination. This implies that the isotopic composition of partial melts and fluids generated in the crust cannot necessarily be predicted from the bulk composition of the crustal source rocks (compare ref. 14). Partial melts and aqueous fluids could easily be richer in ^{87}Sr for example, than the bulk crustal source rocks, because of preferential participation of Rb-rich, Sr-poor phases in the breakdown and melting reactions, and lack of time for complete fluid–residuum or melt–residuum equilibration. Such Sr diffusion data as exists[15] suggests that 5 mm feldspars could remain out of isotopic equilibrium with a crustal melt for 10^3 years or more, considerably greater than the time scale envisaged for production of crustal partial melts or active fluids and their assimilation into the mafic magma. The geochemical patterns resulting from addition to the basalt magma of contaminants which are isotopically unrepresentative of their source rocks may be highly complex. Also, as a magma may undergo contamination at several stages of its passage through the crust, no single mixing relationship is expected.

A feature of Fig. 2 is the length of time for which crustal partial melts and active aqueous fluids persist after basalt intrusion. This may be important because the major barrier to be overcome in contamination via partial melt or fluid phases is penetration through the basalt chill. In a crust which fractures to permit magma intrusion through conduits and fissures, it is unlikely that chills could remain unbroken, because further fracture must often occur after an initial magma route is established, either to admit additional dykes/conduits, or to allow increased flow through those existing. Probably the most favourable situation for contamination to occur is where magmatic pulses follow each other at intervals through the same zone of crust; say every 10–300 years for 100 m fissures. There is then a reasonable chance that new batches of magma will intrude through the heated, mechanically weakened crust adjacent to earlier largely solidified conduits, and assimilate any partial melts and hot fluids that are present. It is a common observation that igneous activity, intrusive or extrusive, is an episodic and repetitive process.

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Early development of Tethys and Jurassic ophiolite displacement

S. J. Haynes

Department of Geological Sciences, Brock University, St Catharines, Ontario, Canada

P. H. Reynolds

Departments of Physics and Geology, Dalhousie University, Halifax, Nova Scotia, Canada

Previous tectonic reconstructions of the Iranian segment[1–4] of eastern Tethys have assumed that the stratigraphic positions of ophiolites from Zagros (late Cretaceous) and Makran (early Tertiary) represent the time of initial oceanic plate displacement. We present ^{40}Ar–^{39}Ar age spectra for mineral separates from metamorphic assemblages associated with the ophiolites of the eastern Zagros and the western Makran, Iran. One of the Zagros samples is from an amphibolite associated with metamorphosed basic and ultrabasic rocks while the other is from metamorphic olistolith within radiolarites, south of the main Zagros ophiolite body. The Makran sample is from a block of amphibolite contained within metamorphosed harzburgites.

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The apparent ages suggest that initial oceanic plate disruption and tectonic emplacement of the Zagros and Makran ophiolites occurred in the middle Jurassic (~170 Myr ago) with further displacements in the late Cretaceous (~97 Myr and ~89 Myr ago). The similarity of the Jurassic age with ophiolite emplacement ages in Yugoslavia[5] and Greece[6] is possible evidence of a middle Jurassic collision of an early Mesozoic Tethyan ridge system with both eastern Europe and central Asia.

In the Neyriz sector of the Zagros, two distinct metamorphic regimes[7] are separated by the Cheshmeh Anjir fault (Fig. 1). North of this fault, late Jurassic–early Cretaceous plutonism and Abukuma-type metamorphism prevail. These are probably related to ascent of thermal flux derived from a descending lithospheric plate[7]. The region south of the fault comprises thrust slices of mixed lithology that contain metamorphosed basic and ultrabasic rocks, and widespread Barrovian-type metamorphic assemblages. In a previous study of this area[7], amphibolites associated with metamorphosed ophiolite assemblages (including spilitic pillow lavas) yielded K–Ar ages of ~170±8 Myr and ~89 ±7 Myr (Fig. 1). These ages were considered minimum values, and a Triassic age of metamorphism was favoured.

The ^{40}Ar–^{39}Ar age spectrum obtained in the present study for biotite from a garnet amphibolite assemblage (H83, Table 1) is presented in Fig. 2. An apparent age plateau was not obtained for this sample. The suggestion of a low age 'saddle' in the intermediate temperature region of the spectrum is indicative of thermal alteration[8,9]. The high temperature age, ~87 Myr, although properly interpreted as a minimum value, is probably close to the true age. The similarity between this and Adib's[7] 89-Myr hornblende value, above, suggests that a distinct metamorphic event occured at this time. Age spectra for biotite and muscovite from psammitic layers in an olistolith (H86, Table 1) are shown in Fig. 2*b*. The spectra are both essentially flat over >90% of the gas release; however, small differences in apparent age (±~1%) are analytically distinguishable. The platform values for biotite and muscovite are respectively 98±1.2 Myr and 96±1.3 Myr. This suggests that a second discrete period of Upper Cretaceous metamorphism occurred at the Iranian plate margin ~97 Myr ago. It confirms a syn- or post-Cenomanian age of emplacement for the main Zagros ophiolite, an event which may have been as late as the early Maestrichtian[2]. Hornblende from an amphibolite enclosed by ultramafic rocks of the western Makran ophiolite (HB1, Table 1) gave a flat ^{40}Ar–^{39}Ar age spectrum within the limits of analytical error (Fig. 2*c*). The plateau age, 170±5 Myr, is middle Jurassic. The final emplacement age of the western Makran ophiolite is uncertain[10] but Falcon[11] has presented evidence for late Cretaceous movements that could be related to ophiolite emplacement and exhumation. During the late Cretaceous–early Tertiary the western Makran ophiolites were displaced southwards from the Zagros along the Zendan fault (Fig. 1), which at that time may have been a transform[12]. The present north-northwest structures in the western Makran (including the ophiolites) were the result of compression and overthrusting from the east along the Zendan Line in the Mio-Pliocene[12]. Adib[7] reports a K–Ar hornblende age of 170±8 Myr for a garnet amphibolite from the Neyriz sector of the Zagros, immediately south of the Cheshmeh Anjir fault (Fig. 1). The agreement between this age and our hornblende age (HB1 Fig. 1) from the western Makran amphibolite indicates that a major middle Jurassic metamorphic/tectonic event affected both the Makran and the Zagros.

Table 1 Sample locations and descriptions

Sample	Locality	Description
H83	Kuh-e Ceghalatun ~10 km south of Gouri, Zagros	Biotite schist layer in garnet amphibolite sequence. Overlain by recrystallised dolomitic marbles. Amphibolite cut by metamorphosed basic and ultrabasic rocks. 7 km west of 89±7 hornblende K–Ar locality where spilitic pillow lavas occur[7].
H86	~8 km west of Neyriz, Zagros	Olistolith of metamorphic rock in radiolarities of the Coloured Series 4 km south-west of the thrust contact of the main Zagros ophiolite body[2]. Olistolith comprises bands of psammites (muscovite, biotite, epidote and piedmontite layers), epidote calc-schists and epidote amphibolites.
HB1	~35 km north-east of Minab, Makran	Amphibolite in contact with serpentised harzburgites and serpentinites of Makran ophiolite. Hornblende, plagioclase with minor biotite. Displays strong lineation parallel to contact. Parallelism of fracture cleavage in serpentinites to contact suggests a thrust relationship.

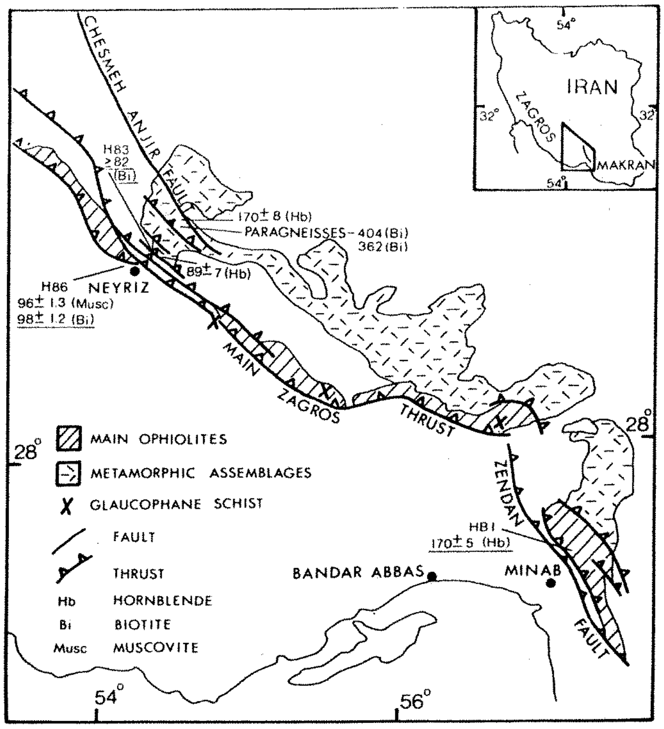

Fig. 1 ^{40}Ar–^{39}Ar ages (underlined) with published K–Ar ages[7] of metamorphic assemblages in ophiolite-bearing thrust slices of the eastern Zagros and western Makran.

Tectonic emplacement models must explain the irregular distribution of amphibolites and Barrovian-type metamorphic assemblages associated with the Zagros and Makran ophiolites which are themselves deformed/metamorphosed (serpentine, rodingites, talc), and their emplacement in a sequence of high-angle thrust slices. We postulate that Barrovian type metamorphic assemblages of the Zagros and Makran result from the frictional heating/pressure effects created during descent of subducting oceanic crust beneath a continental margin; and that spatial relationships of metamorphic facies to the descending plate/continental margin are similar to those proposed by

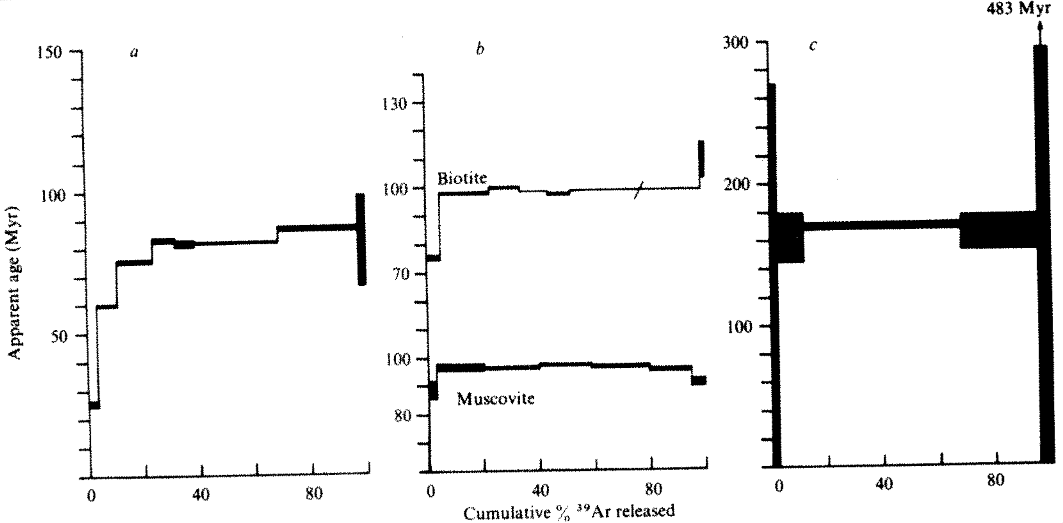

Fig. 2 Age spectra for: *a*, sample H83 biotite; *b*, H86 biotite and muscovite (the small diagonal slash separates two gas fractions); *c*, HB1 hornblende. Half-heights of black bars represent the 2σ relative (between-step) uncertainties. Decay and isotopic constants are those suggested by Steiger and Jäger[14]. Analytical procedures have been described elsewhere[15].

Ernst[13]. Thus, the middle Jurassic (~170 Myr) ages of metamorphism represent the time of initial disruption, uplift and cooling of Barrovian assemblages associated with oceanic crust. In the eastern Zagros younger slices of disrupted oceanic plate and associated metamorphic assemblages were uncoupled and emplaced to the south probably in at least two (at ~97 Myr and ~89 Myr), and possibly more, periods during the Upper Cretaceous. Further work in the western Makran may also reveal similar discrete late Cretaceous episodes. A consequence of our model is that the ophiolite assemblages are now observed as 'cold' intrusions, thrust into supra-oceanic plate continental rise/slope sediments. Because the metamorphism occurred only at/above the subducting oceanic plate which then cooled during its ascent after disruption and uncoupling this final emplacement was terminated in the Zagros and Makran by the close of the Cretaceous period, although the position of the western Makran ophiolites seems to be modified by the Mio-Pliocene westward overthrusting along the Zendan fault. Blueschist facies metamorphism has not been reported in the Neyriz and Minab sectors but glaucophane schists[7] are present in the most southerly (youngest?) thrust slices of the intervening area (Fig. 1). Glaucophane schists may be absent from the oldest (middle Jurassic) thrust slices because these slices contain continental basement assemblages having minimum Devonian, or older, K-Ar ages[7] indicating that these thrusts were located on the Iranian continental margin side of Ernst's[13] zone of blueschist metamorphism.

Amphibolites intimately associated with the ophiolites of eastern Iran, Greece[6] and Yugoslavia[5] all have remarkably similar (middle Jurassic) ages. We believe that this and the evidence for Permo-Triassic rifting in both Greece[6] and south-eastern Iran[10,16] are tectonically significant. The simplest, but not necessarily the only, explanation is to suggest essentially simultaneous middle Jurassic collision of an early Mesozoic Tethyan spreading centre (ridge) with both Europe and central Asia.

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The ecological significance of solar UV radiation on aquatic organisms

John Calkins

Department of Radiation Medicine, University of Kentucky, Lexington, Kentucky 40536

Thorunn Thordardottir

Marine Research Institute, Reykjavik, Iceland

Stratospheric ozone shields the Earth from much of the solar UV–B radiation which is the most biologically injurious component of sunlight. It is possible that both human technologies and natural events, such as reversal of the Earth's magnetic polarity, might lead to significant ozone reductions. The methodology of evaluation of the impact of an agent such as solar UV on organisms or ecosystems has not been firmly established and there has been little research in this area in the past. A new approach is proposed here. If solar UV were an important injurious factor in nature, then living organisms would be required to expend energy to cope with the solar UV exposure they receive. If organisms must specifically mitigate solar UV injury, then it would be expected that only a minimal tolerance would be achieved. We have quantitatively estimated exposure and tolerance of a number of aquatic organisms and find them to be remarkably similar.

Comparison of solar UV effects at high and low latitudes offers opportunities for analysing solar UV as an environmental agent. The visible component of sunlight is not greatly attenuated regardless of the thickness of the atmosphere penetrated, but the UV portion, especially the shorter and more biologically injurious UV–B wavelengths ($\lambda = 280$–320 nm) are strongly absorbed by stratospheric ozone and thus will be highly dependent on the solar angle. Summer solar irradiance reaching Reykjavik (≈ 650 cal cm^{-2} deg^{-1}) approximates the energy reaching temperate or tropical regions. The UV–B radiation at Reykjavik, however, is about half the maximum daily amount reaching temperate and about 40% of that reaching tropical areas. Seasonal effects on UV–B are also much greater at high latitudes.

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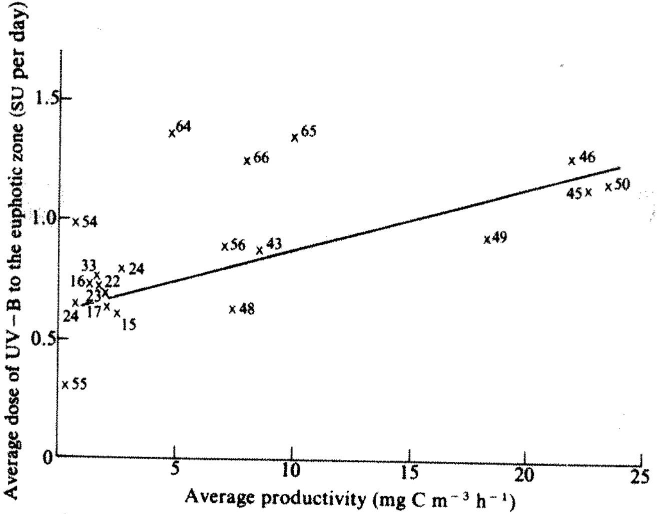

Fig. 1 Typical exposures of marine organisms in the euphotic zone. The average UV – B exposure to the euphotic zone on a sunny summer day, assuming 10 SU incident at the water surface, as measured by the Robertson sensor[3,4]; exposure is plotted as a function of average productivity of the station. Although UV – B transmission varied from station to station, the corresponding changes in visible light transmission tended to maintain a relatively constant exposure in the euphotic zone. Organisms in the more productive euphotic zones receive a somewhat higher UV – B exposure than at stations of low productivity.

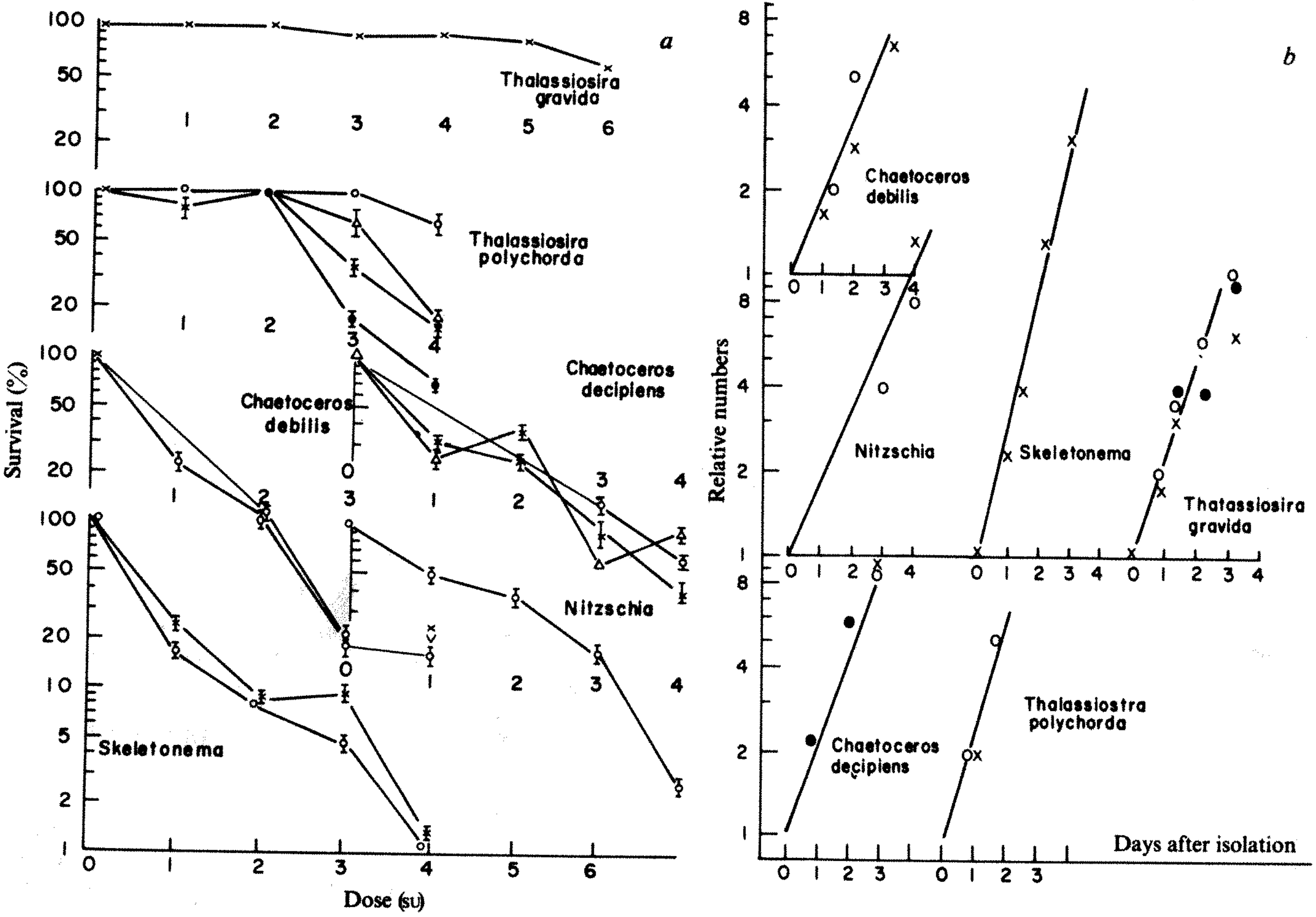

Fig. 2 *a*, The UV – B sensitivity of six marine diatoms. Components of the marine phytoplankton, six common diatoms, were selected as critical organisms for the study of biological response to UV – B. The diatoms were isolated from water samples collected in 1976. *Chaetoceros debilis* and *Chaetoceros decipiens* and *Nitzschia* were derived from collections off the north of Iceland, *Thalassaiosira polchorda*, *T. gravida* and *Skeletonema* were isolated from samples collected near Reykjavik. The diatoms grew well as clones in Erdschribers medium diluted 4 : 1 with seawater; growth was at 7 °C under continuous illumination (1,550 lx) from fluorescent lights. Irradiated and control organisms were isolated into individual depressions containing about 0.25 ml of medium using a micropipette while observing through a low power microscope. The various species of diatoms grow in short connected chains; since the cells of these chains have no protoplasmic connections, but are only linked through inert fibres, we assume that each cell is an independent unit. The various organisms were irradiated with 'simulated' solar UV – B, generated by Westinghouse type FS 20 lamps, and filtered with a Pyrex glass filter of nominal thickness 1/8th inch (ref. 2), monitored using the Robertson sensor; the irradiance from our simulator was 2 SU h^{-1} which approximates the highest irradiance measure in Iceland (1.67 SU h^{-1}). The test organisms were irradiated suspended in medium contained in a small cooled amber plastic dish 2 mm deep, a separate vessel was removed for each dosage and the organisms isolated after the desired exposure times. Two types of controls were run, the 'dark control' was held in a refrigerated incubator at 10 °C in the dark for the time of the longest irradiation (2 h) while a second control, the 'light control', was covered with thick plate glass, thus being exposed to identical conditions except for the UV – B component which was removed by the glass. In all cases the light and dark controls were quite similar in response. The fate of all isolates was followed until the isolated individuals had either died or shown a capacity for sustained growth. A minimum of four post-irradiation divisions was considered to be required to indicate survival. Single cell survival, accounting for control mortality and for multiple organisms in each depression, was computed along the lines previously described[8]. *b*, The growth of marine diatoms. It is assumed that injured populations recover by the growth (division) of survivors, which was determined by repeated counting of isolated diatoms, both unirradiated control organisms and organisms irradiated to various dose levels.

The effects of solar UV – B can be assessed through four independently measurable factors: (1) the amount of solar UV – B incident at the water surface; (2) its attenuation in reaching the position of the various critical organisms; (3) the UV – B sensitivity of the various species comprising the ecosystem; and (4) the capacity of the irradiated organisms to repair injury and replace components of the population killed by UV – B exposure. The four factors have been observed for Icelandic marine ecosystems and compared with similar observations made in Kentucky.[1,2]

As expected, the UV – B irradiance incident on Reykjavik, Iceland (expressed in a biologically weighted unit, the SU)[3,4] reached a peak near midsummer noon (high solar angles), whereas total irradiance was much less dependent on solar angle.

The attenuation of UV – B and visible light was measured at 33 locations in the oceans off Iceland. The productivity of waters from depths of 0, 10, 20 and 30 m was also determined (as ^{14}C incorporation under standard illumination) at many of the stations.

The average irradiance incident in a given volume of water can be calculated using the Morowitz formula[5]. Using the common approximation that the euphotic zone (where photosynthesis > respiration) lies above the depth (Z_{ez}) receiving 1% of the surface irradiance, then the average UV – B dosage to the euphotic zone (during a sunny summer day) can be computed. Figure 1 shows the average UV – B dosage to the euphotic zone plotted against productivity.

The action of UV – B exposures of 0.5–1.5 SU (Fig. 1) can be established by examining the effect of such exposures on key organisms in the marine ecosystem. Figure 2*a* shows the survival response of the six diatom species irradiated with simulated solar UV – B. While *Thalassiosira* spp. were relatively resistant, the other four diatoms showed substantial killing at a radiation dose of 1 SU.

The maximum tolerable exposure to various agents for organisms multiplying asexually can be estimated[2] by determining the replacement limiting dose (RLD) for a species, which is the daily exposure which would kill a fraction of the population just equalling the daily growth capacity of the survivors.

Figure 3 shows the RLD values of various organisms, together with the exposure which they might typically receive. Computed exposure often equals or exceeds the tolerance of organisms; however, the ability of organisms to survive such an apparent excess exposure can be explained on a case by case basis. *Escherichia coli* shows a RLD less than exposure[1], although it is known to have three separate pathways of DNA repair, all of which reduce its sensitivity to sunlight[6]. It might seem that *E. coli* needs to be able to withstand sunlight; however, *E. coli* is well known to 'die off' in natural waters. Because *E. coli* spends most of its life cycle in the sunlight-protected intestine of host animals, species propagation may require short-term survival in natural waters, living only long enough to transfer from host to host.

Some protozoans and arthropods show apparent tolerance levels below the exposure levels in shallow waters (Fig. 3). *Paramecium aurelia* in particular tends to remain at the surface; however, it also shows a preference for shielded surface locations[7] (such as rocks and plants protruding from the water). Another protozoan, *Coleps*, shows a pronounced avoidance of light by moving deep into the water column[7]. Arthropods, *Cyclops*, *Cypris* and *Daphnia*, have also been investigated. *Daphnia* can reach reproductive maturity 10–15 days after birth and produce broods of about five offspring at 10-day intervals thus showing a growth rate of 1.38 per day. RLD would equal 72% survival or 2 SU per day. *Daphnia*, *Cyclops*, and the sensitive protozoa show avoidance of UV – B exposure, coping by behaviour, while the very radiation resistant *Cypris* (RLD > 20 SU) seems to seek intense illumination[7].

Some algae also seem to fall short of the required minimum tolerance. One filamentous freshwater alga, *Rhizoclonium*, floats on ponds during the summer and would seem to require tolerance greater than the 8 SU observed. The surface mat was heavily pigmented and senescent while new growth extended downwards and was protected by the surface layer. This organism seems to reach the required UV – B resistance in the same way as many higher organisms, through specialised protective organs or tissues. Although we lack quantitative data to explain the sensitivity of marine diatoms, they change buoyancy in response to illumination, rising with visible light and falling after UV – B exposure. It is possible that they and unicellular freshwater algae obtain adequate photosynthetic light with minimal UV – B exposure by rising in early morning and late evening and sinking for UV – B protection at midday when UV exposure is greatest, a movement pattern often observed in algae.

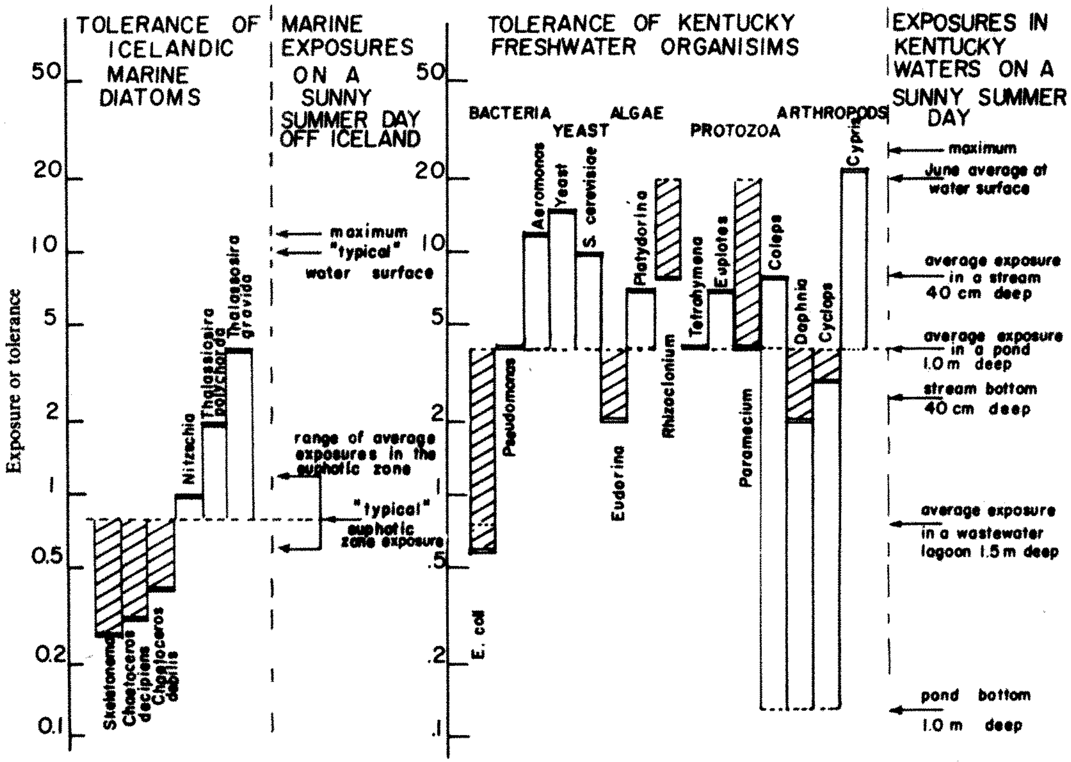

Fig. 3 A comparison of exposure and RLD. Maximum tolerable doses (double bars) are shown for a number of aquatic organisms together with the level of exposure for typical locations where the organisms are found. When exposure is less than tolerance the difference is indicated by solid bars; when exposure exceeds tolerance the difference is indicated by crosshatching. The apparently paradoxical situations where organisms lack sufficient resistance to live in the areas where they are, in fact, found is considered on a case by case basis in the text.

Our findings may be compared with similar calculations made for environmental ionising radiation. The RLD for 'wild-type' *E. coli* is 5–30 krad per day, almost 10^8 times the natural exposure (0.1 rad per yr); for *Tetrahymena pyriformis* the ionising radiation RLD is more than 10^9 times exposure. The ionising radiation resistance of aquatic microorganisms can hardly be an adaptation to a direct stress; resistance to ionising radiation depends on genetic characteristics which could be lost through mutation; resistance to ionising radiation must be a by-product of other requirements, perhaps resistance to solar UV, since the two forms of radiation resistance are highly correlated.

The finding that tolerance and exposure to solar UV are approximately equal strongly implicates solar UV as a significant ecological factor, suggesting that there may be no large reserve of resistance which could cope with altered solar UV exposure without requiring modification of physiology or behaviour. Doubtless most organisms would adapt if the solar UV were to increase. Organisms can increase pigmentation, repair capacity, or avoid solar UV; however, both physiological and behavioural adaptation to solar UV reduces the resources available for other purposes.

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LH-RH in the mesencephalic central grey can potentiate lordosis reflex of female rats

Yasuo Sakuma & Donald W. Pfaff
The Rockefeller University, New York, New York 10021

The lordosis reflex, which characterises female reproductive behaviour in rodents, can be potentiated in oestrogen-primed ovariectomised female rats by electrical stimulation of the ventromedial nucleus of the hypothalamus (VMN)[1]. Subsequent demonstration of an immediate and large facilitation of the lordosis reflex from the mesencephalic central grey (CG)[2] prompted us to speculate that the VMN may exert an oestrogen-dependent tonic bias on the mesencephalic circuitry for lordosis via its heavy descending projections[3]. Moreover, we considered that luteinising hormone-releasing hormone (LH-RH) could be involved in this system. LH-RH can potentiate lordosis when given systemically to oestrogen-primed ovariectomised rats[4,5], and neurotropic effects of this peptide have been shown by microiontophoresis[6,7]. Also, some axons in the CG have been stained with antiserum to LH-RH[8]. The present study examined a possible role of LH-RH in the CG in the regulation of the lordosis reflex of oestrogen-primed ovariectomised female rats. Infusion of exogenous LH-RH in the CG had an immediate facilitative effect on the lordosis reflex, whereas passive immunisation against endogenous LH-RH by anti-LH-RH γ-globulin diminished the reflex.

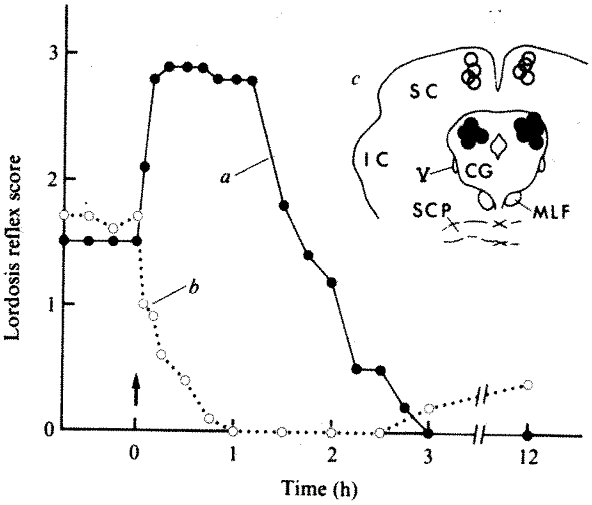

Fig. 1 Successful modification of lordosis reflex performance by LH-RH (*a*, ●) and anti-LHRHG (*b*, ○) in rat no. 6. Infusions were made at 0 h (arrow). *c*, Effective sites of infusions in five rats (●) were in the central grey (CG). Infusions in the superior colliculus (SC, ○) in four rats were ineffective. The diagram was taken from a frontal section of the mesencephalon 6.0 mm posterior to the bregma. Abbreviations: IC, inferior colliculus; MLF, medial longitudinal fascicle; SCP, superior cerebellar peduncle; V, mesencephalic nucleus of the trigeminal nerve.

Intracerebral infusion was accomplished in ovariectomised Sprague–Dawley female rats chronically implanted with stainless steel 22-gauge guide cannulae bilaterally in the CG. The animals received a subcutaneous injection of 5 μg of oestradiol benzoate in sesame oil. They were given a screening test 96 h later, and those which exhibited moderate lordosis in response to manual cutaneous stimulation of the flanks followed by rump-tail base-perineum region[9] were selected. The infusion was made with internal cannulae: 28-gauge stainless steel tubes fitted into the guide cannulae and protruding 0.5 mm from the tip of the latter. LH-RH (Ayerst) was dissolved in saline in a concentration of 100 μg ml^{-1}. Lyophilised sheep anti-LH-RH γ-globulin (anti-LHRHG) and normal γ-globulin (NSG) were dissolved in saline to reconstruct the original concentration in the serum. Each solution was prepared immediately before use, and was infused over a period of 60 s, in a volume of 0.5 μl through each cannula. Two sets of inner cannulae and microsyringes were used for consecutive delivery of the fluid into the bilateral cannulae. Placement of the internal cannulae and the infusion were carried out with the animal in a restrainer but not anaesthetised. The lordosis performance of an animal at a given time was assessed by applying manual cutaneous stimuli five times, at brief intervals. Lordosis reflex strength with each stimulus application was rated using a scale from 0 (no vertebral dorsiflexion) to 3 (strongest possible response), and the average of five ratings was calculated. This average, which we termed the lordosis reflex score, provided virtually identical results when collected independently by two investigators. It has been confirmed that animals with a high reflex score consistently show high receptivity with male rats[1,2]. The lordosis score was usually determined at intervals of 5–30 min during the experiment. Following the completion of observations, tip positions of the internal cannulae were determined in frozen serial sections (100 μm) stained with luxol fast blue and cresyl violet.

Injection of LH-RH into the dorsal part of the CG produced a prompt facilitation of lordosis which was comparable to the effect of electrical stimulation[2]. For example, significant effects could be repeatedly produced with similar time courses in five animals. Facilitation caused by these infusions occurred within 5 min of the end of each application and lasted for about 2 h in each case. Figure 1*a* shows an experiment depicting the time course of the facilitation. This facilitation was followed by prolonged suppression of the reflex in three of the five rats.

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Increased grooming and licking behaviour often accompanied the facilitation of lordosis, and mere brief contact on the fur of the flanks was sufficient to trigger lordosis in these animals. An equivalent volume of physiological saline had no significant effect when infused into the CG, and infusion of LH-RH into the cerebral cortex also failed to produce any effect.

Passive immunisation with anti-LHRHG resulted in a complete loss of the lordosis reflex when infused into the CG of the same animals. The reflex disappeared within 90 min after the infusion. The suppression of the lordosis reflex persisted for as long as 12 h (Fig. 1*b*). The lack of aggressive behaviour (often elicited in non-receptive female rodents by somatosensory stimuli[10]) was noted when these animals were made unresponsive by anti-LHRHG. NSG injection in the CG had no apparent effect on the performance of lordosis.

Effective sites for the infusions of LH-RH and anti-LHRHG were located in the dorsal half of the CG. Neither LH-RH nor anti-LHRHG had an appreciable effect on the lordosis reflex when infused in the superior colliculus (Fig. 1*c*).

LH-RH bioactivity has been reported to be present in the mesencephalon of the dog and rabbit[11]. By immunohistochemical techniques, some axons in the CG[8] of the guinea pig stain with antiserum to LH-RH. These extra-hypothalamic LH-RH axons have now been implicated in the control of reproductive behaviour. Although a neurotropic effect of LH-RH has not been shown for CG neurones, neurones in the medial basal hypothalamus are either excited or inhibited by microiontophoresis of LH-RH[6,7]. Because electrical stimulation of the CG effectively facilitates the lordosis reflex in oestrogen-primed ovariectomised female rats[2], it is possible that endogenous LH-RH has excitatory effects on the CG neurones involved in the control of lordosis. The origin of mesencephalic LH-RH axons has not been identified, but a contribution of cells in or near VMN cannot be ruled out. LH-RH activity has been detected in the lateral part of the VMN[12]. If this is the case, the requirement for oestrogen in the successful electrical facilitation of lordosis from VMN[1] may be explained at least in part by a requirement for hypothalamic LH-RH[13], which can be mobilised by electrical stimulation. In this context, it may be significant that both LH-RH infusion and VMN stimulation[1] caused long-lasting facilitation of lordosis after each manipulation.

Earlier attempts using infusions of LH-RH[14] or anti-LHRHG[15] to the brain suggested modulatory effects of these substances on the performance of lordosis. With combined observations on the effects of LH-RH and anti-LHRHG, we have now demonstrated that a hypothalamic hormone is necessary for a given behaviour. Blocking the action of decapeptide LH-RH could block lordosis behaviour. In turn, an exact site in the brain where LH-RH is required, the midbrain central grey, was revealed by the location of the microinfusion cannula tips. The identification of a specific neurochemical (LH-RH) needed for reproductive behaviour and a particular site of action may allow specific interference with mating behaviour, with other behavioural responses left undisturbed.

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Paternal investment of urates in cockroaches

Donald E. Mullins & Clifford B. Keil

Department of Entomology, Virginia Polytechnic
Institute & State University, Blacksburg, Virginia 24061

During copulation, the male German cockroach [*Blattella germanica* (L.)] deposits a spermatophore in the female's genital pouch and voids stored urates from his accessory glands (uricose glands) on to the spermatophore[1]. About 24 h after mating, the female expels the empty spermatophore with adhering urates. Although uricose glands have been reported in the Blattaria[2,3], and are proposed to act in excretion[4,5], their role in reproduction is unclear. Roth[2] has suggested that urates may serve to protect the spermatophore from premature consumption by the female or other insects. However, we have noted the disappearance of spermatophores/urates from cages containing mated pairs of cockroaches and have investigated the possibility that these materials have nutritional value. We observed that spermatophores and urates disappeared between 7 and 18 d after females were mated while on a dog food diet; spermatophores were consumed within 4 d by females starved for 3 d after eclosion. Furthermore, we now report that labelled uric acid assimilated by males before, and voided at, mating can be recovered in mated females and their ootheca.

Accessory glands containing high levels of labelled urates were obtained by injecting males with [8-^{14}C]hypoxanthine (Table 1). One of each labelled pair of males mated successfully and after 38 d all females and their oothecae were radioactive. Table 1 shows that females on a low protein (5%) diet

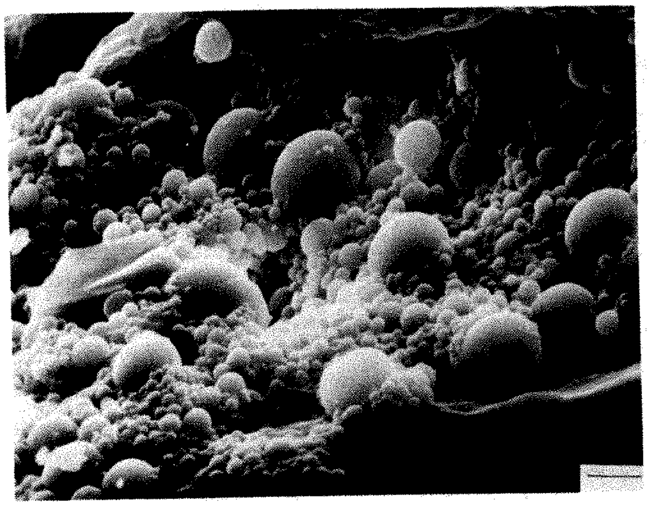

Fig. 1 A scanning electron micrograph (SEM) of the edge of an emptied spermatophore with adhering urate spherules which are secreted/deposited by males at the time of mating. These urates may be consumed (along with the spermatophore) and used by the female and/or be subsequently incorporated into the ootheca as a nitrogen resource. Before viewing, the specimen was coated with a 60:40 mixture of gold/palladium. It was examined with an Advanced Metals Research SEM (Model 900-52) equipped with EDAX (energy dispersive X-ray analysis, Model 707A). Scale bar, 5 μm.

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Table 1 Fate of labelled ^{14}C-hypoxanthine injected into young adult male *B. germanica* which were subsequently presented to young adult females

Diet	Whole insect/oothecal activity in (d.p.m.)			
	Females	Oothecae	Mated males*	Unmated males
5% Protein	2,073 ± 961 *a*†	9,417 ± 2,725 *b*	51,681 ± 9,181 *c*	103,948 ± 3,688 *d*
25% Protein	2,486 ± 704 *e*	800 ± 275 *f*	70,635 ± 4,256 *g*	107,746 ± 3,905 *h*

Adult males (1–3 days old reared on dog food) were injected with 1 μl[8-^{14}C]hypoxanthine, resulting in a measured 181,418 ± 6,697 d.p.m. per insect. They were maintained on 25% casein protein diet for 10 days, followed by presentation of two males (see below) to one young virgin female (reared on dog food) in a plastic Petri dish supplied either with a 5% protein or a 25% casein protein diet and water. In addition, unmated males (maintained on 25% casein protein) were frozen 10 and 48 days after isotope injection. Stock cultures, rearing conditions and diet formulations used are described elsewhere[12,16,17]. Before extraction, insects and oothecae were rinsed several times in 0.6% Li_2CO_3 at 30 °C to remove any labelled material adhering to their exteriors. Whole insect assay included homogenising whole bodies in 0.6% Li_2CO_3 at 80 °C for 10 min, centrifugation at 1,000*g* for 10 min and counting in PCS (Amersham/Searle)/xylene scintillation cocktail. Oothecae were extracted in 0.1 M Tris-HCl, buffer *p*H 7.4, at 30 °C, centrifuged and counted as above. A portion of these extracts was frozen for subsequent analysis (Table 2). Data represent the mean ±s.e.m. ($n = 8$ females per diet). Ten days after injection virgin males contained 124,279 ± 8,443 d.p.m. per insect and after 48 days they contained 107,908 ± 2,203 d.p.m. per insect ($n = 4$). Accessory glands contained 57,989 ± 14,066 and 36,365 ± 1,644 d.p.m. per mg ^{14}C-urate in tissue obtained 10 and 48 days after injection, respectively ($n = 4$). Purification of these extracts indicated that virtually all the activity in this tissue could be attributed to labelled urate. One discarded spermatophore/urate obtained from a mated pair contained a total activity of 54,976 d.p.m. in the extracted urate fraction. ^{14}C-hypoxanthine was used instead of xanthine or uric acid because of its greater solubility in aqueous solutions; this facilitated the process of injecting relatively constant amounts in each insect. The subsequent conversion of hypoxanthine to uric acid stored as urates in the males proved to be quite suitable for our purposes.

* Virgin females were provided with two males to allow an optimal opportunity for mating to occur. In these conditions only one male will normally mate successfully[18], presumably releasing its urate stores. We have arbitrarily segregated the males on the basis of whole body activity, hence 'mated males' were compared with 'unmated males'. These differences are highly significant (see below).

† Statistical analysis using the Student's *t*-test indicated the following: $a = e, P < 0.7321$; $b \neq f, P < 0.0152$; $c \neq g, P < 0.082$; $d = h, P < 0.4868$; $c \neq d, P < 0.0001$; $g \neq h, P < 0.0001$.

incorporated more activity into their oothecae than did those maintained on 25% casein protein diet. The amount of label consumed and transferred to the ootheca varied considerably. Presumably, this variation reflected the amount of label excreted by males, the feeding activity and nutritional status of females. Obviously, these radioactivity transfer estimates could have been influenced by metabolism of urates to $^{14}CO_2$, which was not measured.

The disappearance of deposited spermatophores/urates suggests that the major route of urate entry into females is the alimentary canal. This does not preclude the possibility that a small fraction may enter by the genital tract[6]. Radioactivity within oothecae was mainly in the soluble fraction, with very little in the precipitated protein or oothecal cases (Table 2). Analysis of the soluble fractions indicated that the major radioactive component was uric acid. Urate content was examined in oothecae at different stages of embryonic development and newly hatched nymphs. Newly formed oothecae contained 2.8 ± 0.1 μmol uric acid and newly hatched 1st instar nymphs plus their empty oothecal cases contained 1.2 ± 0.1 μmol. Correlation analysis showed a significant decline in uric acid levels during embryogenesis ($y = -0.056x + 2.72$; Pearson's $\rho = -0.789$; $P < 0.0001$). This decline strongly suggests that uric acid is metabolised during embryonic development.

Large nutritional demands are made on the metabolism of the female German cockroach during oothecal production. Kunkel[7] has shown that the female incorporates almost 90% of her food reserves accumulated during the preovipositional period into a single ootheca. A portion of these reserves are nitrogen-containing materials which are transferred to the oocyte in the form of vitellogenin, appearing in the female blood 3 or 4 days after adult ecdysis and disappearing soon after terminal oocytes have been released into the ootheca[8]. It now seems that females are also incorporating uric acid into developing oocytes. Because uric acid levels decrease significantly as the embryo develops, it is likely that this incorporated uric acid is metabolised during embryogenesis.

Another event occurring during oogenesis is trans-ovarian transmission of symbiotic bacteroids (mycetocyte bacteroids) from females to the oocytes[9]. Although the precise role of cockroach mycetocyte bacteroids is not clearly understood, they have been implicated in amino acid, urate and vitamin metabolism[9–12]. It is possible that these bacteroids might play an important part in the metabolism of oothecal uric acid.

There have been several reports that female Orthoptera consume a spermatophore which has been ejected after mating, and it may not be uncommon for a male to transfer materials of nutritional value in the accessory gland secretions[6,13,14].

Gordon[15] has characterised cockroaches as being versatile omnivores capable of surviving on foods of low quality and limited quantity. Also, those nutrients most likely to be stored are those which may be of limited supply in the environment. Storage of urates (which are generally viewed to be end products of nitrogen metabolism and discarded by most higher uricotelic organisms) as a nitrogen resource by organisms adapted to utilise them in conditions of dietary stress has obvious advantages[12].

We suggest that male *B. germanica* urate secretions (Fig. 1) may represent paternal investment of a nitrogen resource from which the female and her progeny might benefit. The evidence presented that urates secreted during mating are incorporated into ootheca at various levels related to the dietary nitrogen status of the parents, and the discovery that uric acid is present in newly formed oothecae emphasise the importance of this material to this group of insects. However, little is known about catabolism of uric acid. Studies using ^{14}C-labelled purine intermediates reflect only pathways involving the carbon atoms and do not necessarily reflect the path of nitrogen contained in

Table 2 Distribution of radioactivity in whole oothecae obtained from female *B. germanica* which had been paired with labelled males for 38 days

Diet	Soluble fraction		Precipitated fraction	Oothecal cases	Total
	d.p.m.	Uric acid*	d.p.m.	d.p.m.	d.p.m.
5% Protein	9,470 ± 2,751	58%	88 ± 18	163 ± 60	9,721 ± 2,790
25% Protein	727 ± 314	88%	15 ± 3	22 ± 8	760 ± 323

Frozen oothecal extracts obtained from the experiment described in Table 1 were heated, centrifuged and the supernatant (soluble fraction) counted in PCS/xylene solution. The precipitated pellet was resuspended and washed several times before suspension in the counting solution. The oothecal cases were extracted several times in heated buffer and ground to a very fine powder before placement in counting solution. The per cent uric acid content was obtained from pooled samples of the soluble fractions (5% protein and 25% casein protein) after purification by TLC (1-mm cellulose plates developed in *n*-butanol/methanol/H_2O/60 : 20 : 20 : 1) and scintillation counting.

* Uric acid present in the urate spherules excreted by the males is partially potassium urate, hence we refer to it as urate. However, because nothing is known about its form in the ootheca, we refer to it as uric acid.

urates. Therefore, investigations designed to elucidate these relationships further might prove useful.

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Chronic blockade of dopamine receptors by antischizophrenic drugs enhances GABA binding in substantia nigra

Karen Gale

Department of Pharmacology, Georgetown University Schools of Medicine and Dentistry, 3900 Reservoir Road, Washington, DC 20007

Antischizophrenic drugs exert their primary effects through blockade of dopamine (DA) receptors in the central nervous system (CNS)[1-3]. However, because of intricate interconnections between neural pathways, alteration of DA function in the CNS can lead to changes in the function of other neurotransmitters. In the basal ganglia, the turnover and utilisation of several neurotransmitters, including γ-aminobutyric acid (GABA)[4-6], are indirectly influenced by nigrostriatal DA activity[7-10]. However, although attention has been focused on changes in DA receptors[11,12], relatively little is known about changes in receptors for other transmitters after chronic antischizophrenic drug treatment. It is likely that changes occurring one or more synapses removed from the drugs' primary site of action may account for some of the time-dependent changes in the therapeutic and adverse clinical effects of antischizophrenic drugs[13,14]. In the substantia nigra (SN), a reduction in GABAergic inhibitory tone and a possible increase in the release of excitatory transmitter such as substance P are effects which seem to accompany the acute blockade of striatal DA receptors[15]. Recent studies have shown that a chronic decrease in GABA function in the SN, through the destruction of striatonigral pathways[16,17], produces an increase in the number of GABA binding sites in the SN[14,15]. Therefore, if a relative deficit of GABA transmission in the SN were to be maintained by chronic antischizophrenic drug treatment, an increase in nigral GABA binding might occur. To investigate this possibility, two classical prototype antischizophrenic drugs (chlorpromazine and haloperidol) were examined for their effects on specific GABA binding in the SN and striatum of rats. These effects were compared with those of clozapine, an antischizophrenic drug with an atypical biochemical and clinical profile[4,18-23]. The data obtained, and reported here, support the hypothesis that chronic blockade of dopamine receptors by classical antischizophrenic drugs enhances GABA binding in the SN. This effect may be related to the extrapyramidal side effects associated with classical antischizophrenic drug treatment[13].

Male Sprague–Dawley rats, weighing 200 g at the start of the experiments, were treated for 8 weeks with daily subcutaneous (s.c.) injections of haloperidol (0.7 mg per kg), chlorpromazine (20 mg per kg) or clozapine (20 mg per kg). At the end of the 8-week period, 5 days elapsed without drug administration before the rats were killed for measurement of ^{3}H-GABA binding. Binding measurements were made in a crude synaptosomal–mitochondrial membrane preparation which had been frozen and thawed, treated with Triton X-100 (0.01% for 60 min at 37 °C) and washed extensively[17,24,25].

Table 1 shows the amount of specific ^{3}H-GABA binding in the SN of drug-treated groups. When compared with values from control rats which received daily injections of vehicle, the values obtained from rats receiving either chronic chlorpromazine or haloperidol treatment were significantly elevated. In the same rats in which this increase in specific ^{3}H-GABA binding was found in the SN, no change in specific ^{3}H-GABA binding was obtained in the striatum (Table 1). In contrast, chronic clozapine treatment did not cause a significant change in the specific binding of ^{3}H-GABA in the SN. No changes in specific GABA binding were observed in the SN after acute drug administration (1 h before killing), indicating the importance of chronic treatment for producing an increase in nigral GABA binding. The SNs from several chronically treated rats were pooled for binding studies using a range of GABA concentrations (10–90 nM). Scatchard analysis revealed that the increased GABA binding in the SN of chlorpromazine- and haloperidol-treated rats was due to an increase in B_{max} and not to a change in affinity (K_D) of the binding sites for GABA (see Table 1 legend).

Thus, the classical antischizophrenic drugs, chlorpromazine and haloperidol, although not acutely changing nigral binding sites for GABA, on repeated administration can cause an increase in the apparent number of these binding sites. As GABA binding in the striatum did not change, the increase observed in the SN does not represent a general response of brain GABA receptors to these drugs but probably reflects the juxtaposition of specific neural pathways. As in the case of lesions of striatonigral GABAergic projections[16,17], the increase in nigral GABA binding after chronic drug treatment may represent a compensation for a decrease in the synaptic activity of GABA. This possibility is consistent with the evidence that chronic treatment with haloperidol or chlorpromazine reduces the turnover rate of GABA in the SN[4].

The lack of effect of chronic clozapine treatment on GABA binding in the SN is probably related to the unique pharmacology of this compound. Clozapine does not cause catalepsy in animals and is devoid of Parkinson-like extrapyramidal side effects in man[18,19]. There is evidence that the DA receptors affected by clozapine may be functionally distinct from those affected by haloperidol and chlorpromazine[20-22]. In addition, clozapine's blockade of muscarinic cholinergic receptors[23] may interfere with this drug's ability to activate the striatonigral pathways required for the suppression of GABAergic transmission in the SN. Thus, acute clozapine treatment, unlike haloperidol or chlorpromazine, causes an increase in the turnover rate of GABA in the SN[4].

To evaluate the possible significance of an alteration in nigral GABA receptors, the functional role of these receptors must be considered. Activation of GABA receptors in the SN can prevent the stimulation of tyrosine hydroxylase and DA synthesis which occurs in the striatum after an acute injection of haloperidol[26]. Thus, an increase in SN GABA receptors after chronic haloperidol and chlorpromazine may contribute to the gradual decline in the ability of these drugs to enhance DA turnover[22,27-30]. In addition, non-DA pathways originating in the SN zona reticulata are contacted by GABA terminals[31] and probably contain GABA receptors[32,33]. These latter pathways may mediate the DA-independent behavioural responses (contralateral rotation or stereotypies) evoked by the direct unilateral or bilateral activation of nigral GABA receptors in the rat[34-38]. Stimulation of nigral GABA receptors can also

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Table 1 ^{3}H-GABA specific binding (fmol per mg protein) in SN and neostriatum after chronic treatment with antischizophrenic drugs

Chronic treatment (mg per kg, s.c.) (8 weeks)	SN†	Striatum†
Control	382±15	322±13
Haloperidol (0.7)	501±32* (131)	331±18 (103)
Chlorpromazine (20)	521±30* (136)	348±13 (108)
Clozapine (20)	360±19	338±15 (105)

Values represent the means of 15 rats (3 separate experiments, 5 rats each) ±s.e.m. Rats were killed by decapitation and the SN and striatum dissected out as previously described[15] and placed on dry ice. For each SN determination, both SNs (8 mg wet weight tissue) from one rat were combined; this yielded enough protein (300–400 μg in the final membrane preparation) to run triplicate samples for binding. Tissue was prepared according to the method of Enna and Snyder[24]; as described by Toffano *et al.*[25], this preparation results in the removal of a membrane-bound protein inhibitor of high-affinity GABA binding. Binding was measured at 0 °C in a Na^+-free medium in the presence of 30 nM ^{3}H-GABA; nonspecific binding was measured in the presence of 10^{-3} M nonradioactive GABA[24]. The SN from groups of three or four rats were pooled to obtain values for Scatchard plots of high-affinity binding (10–90 nM ^{3}H-GABA)[17]. In these conditions, linear regression analysis of the data indicated that the K_D values of haloperidol (42±5 nM) and chlorpromazine (40±5 nM) rats were not significantly different from those of controls (36±4 nM). Values of B_{max} (pmol per mg protein) were: control, 1.19±0.06; haloperidol, 1.61±0.10*; chlorpromazine, 1.58±0.12*.

* Values significantly different from controls, $P < 0.05$.

† Numbers in parentheses refer to % of control.

reverse the catalepsy induced by haloperidol[36]. These data suggest that GABA receptors in the SN, functioning downstream from the influence of DA neurones, may participate in the relay of neural output from the basal ganglia.

From this perspective, it is tempting to speculate that a modification of nigral GABA receptors, produced by classical antischizophrenic drugs, may be responsible for some of the behavioural consequences of their long-term administration. Thus, the development of tolerance to catalepsy[27] and the gradual increase in the ability of DA agonists to induce stereotyped behaviour[39,40] in animals, as well as the disappearance of parkinsonian symptoms and the appearance of dyskinesias in humans[13], are examples of processes which occur in response to chronic treatment with antischizophrenic drugs, and which may be brought about by 'supersensitive' receptors, not only for DA, but also for GABA.

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Effects of 4-aminopyridine on normal and demyelinated mammalian nerve fibres

R. M. Sherratt, H. Bostock & T. A. Sears

Sobell Department of Neurophysiology, Institute of Neurology,
The National Hospital, Queen Square, London WC1N 3BG, UK

We have previously demonstrated that a drug which prolongs action potentials can, like a reduction in temperature, overcome conduction failure in demyelinated nerve fibres[1]. Although the particular substance then used, a scorpion venom, was not a suitable therapeutic agent, we suggested that other drugs, with similar but milder effects on the action potential, might be effective in the symptomatic treatment of multiple sclerosis[2]. We now report some encouraging results obtained with 4-aminopyridine (4AP), a substance which blocks the voltage-dependent potassium currents in squid giant axons[3]. The use of a potassium-blocking agent to prolong action potentials may seem surprising because we previously found tetraethylammonium chloride (TEA), another potassium blocker, ineffective on normal rat myelinated fibres[1], and two recent voltage-clamp studies have confirmed that mammalian nodes have few, if any, potassium channels[4,5]. On the other hand, 4AP strongly potentiates transmitter release from the unmyelinated terminals of rat motor nerves[6], and the possibility arose that demyelinated axon membrane, which can conduct impulses continuously like an unmyelinated fibre[7], might further resemble its unmyelinated terminals by responding to 4AP. In testing this hypothesis, we have found that both TEA and 4AP prolong action potentials of demyelinated and unmyelinated fibres, and both facilitate conduction in fibres blocked by demyelination. 4AP is effective at lower concentrations, and is the more promising for clinical use, as it has already been used with beneficial effects in the treatment of Eaton-Lambert syndrome[8] and myasthenia gravis[9].

The contrasting effects of 4AP on normal A and C fibres are most readily seen by comparing the effects on the A and C elevations of the same dorsal root compound action potential. To obtain the records in Fig. 1, a rat was anaesthetised with urethane, and after laminectomy a lumbar dorsal root was raised on stimulating and recording electrode pairs in a paraffin pool maintained at 37 °C. The compound action potentials evoked at low and high stimulus strengths were recorded monophasically from near the cut central end. At low stimulus strength, the 4AP had no effects on the main A-fibre spike (although at high gain the after potential became more negative); the C-fibre

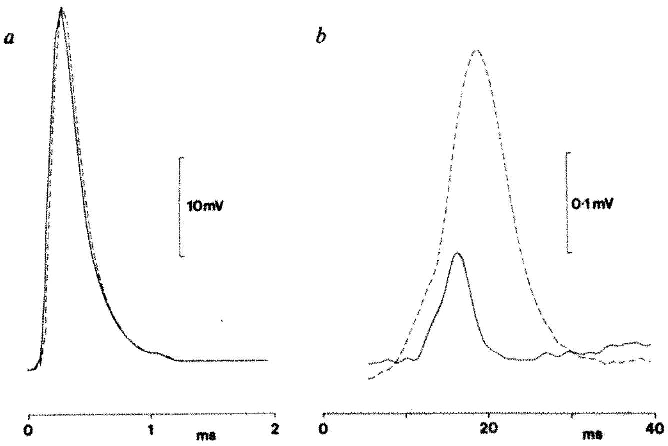

Fig. 1 Effect of 4AP on compound action potential in rat dorsal root, showing myelinated (*a*) and unmyelinated (*b*) fibre components separately. Each trace is the mean of eight, obtained with a digital signal averager. Solid lines, control; dashed lines, following application of 4 mM 4AP.

component, however, obtained by subtracting the low stimulus response from the high stimulus response, was increased in area severalfold. Of 17 dorsal roots treated with 5 mM 4AP, the increase in peak height of the C component ranged from 4% to 550%, with a median of 127%.

To determine whether the change in the C-fibre compound action potential was due simply to a prolongation or to some other change in the individual C-fibre action potentials, and to determine the effect on demyelinated axons, it was necessary to record from single fibres. Figure 2 shows typical examples of the effects of 4AP on the action potentials of single myelinated (*a*), unmyelinated (*b*) and demyelinated (*c*) nerve fibres, also recorded from rat spinal roots. In *b*, the single fibre was stimulated and recorded as in Fig. 1, and judged to be unmyelinated from its conduction velocity of 0.43 m s^{-1} at 30 °C. It was functionally isolated from the myelinated fibres in a dorsal root filament by latency, and from the other unmyelinated fibres by its threshold. In *a* and *c* the action potentials of single fibres in sacral ventral roots were isolated by adjusting the position and intensity of a stimulus to the tail. In *c* the demyelination was caused by intrathecal injection of diphtheria toxin 12 d previously[7]. In each case digital averaging was used to improve the signal-to-noise ratio, and in *a* and *c* the averager sweep was triggered by the spike as described previously[7]. As with the compound dorsal root A spike, the single ventral root myelinated fibre action potential was unchanged by 5 mM 4AP. The single unmyelinated fibre action potential, on the other hand, was prolonged and increased slightly in amplitude. The demyelinated fibre had an action potential of irregular shape, as is usual, and this was also prolonged.

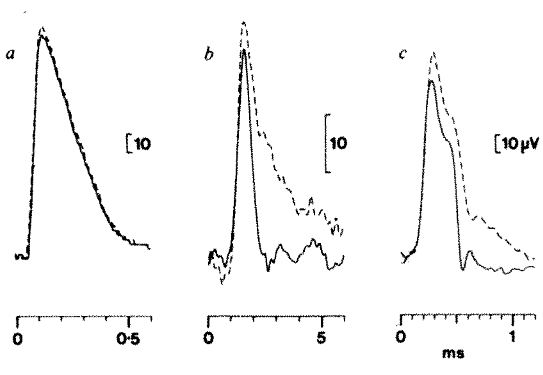

Fig. 2 Effects of 4AP on single fibre action potentials. *a*, Myelinated (37 °C); *b*, unmyelinated (30 °C); *c*, partially demyelinated fibre (37 °C). Each trace is the mean of 64. Solid lines, controls; dashed lines, following application of 5 mM 4AP.

The mechanism of action potential prolongation is best revealed by records of membrane currents, which can be calculated by subtracting adjacent records of longitudinal current, made between closely spaced pairs of electrodes[10]. Membrane current starts in the outward direction as the approaching action potential depolarises the membrane, and in myelinated axons only becomes inward at the nodes, when they are excited. Figure 3 shows the effects of 4AP on the membrane currents at two consecutive nodes in a dorsal root fibre exposed to diphtheria toxin 6 d previously. Conduction in this fibre was saltatory, except for one demyelinated internode, over which conduction was continuous, at only 1.1 m s^{-1} (30 °C). The abnormally large and slow currents in this region resembled those described previously for continuously conducting internodes[11]. The node in Fig. 3*a* was unaffected by the toxin, judging from the small amount of outward current required to depolarise it to threshold, and the 4AP had little effect. The currents in Fig. 3*b* were recorded from the next 'node' 1 mm away, which was also the start of continuous conduction. As at succeeding electrode positions in the demyelinated internode, the membrane currents had three conspicuous phases: outward current during passive depolarisation, active inward current and a late outward current phase. This late phase of outward current, occurring at the time an active potassium current would be expected, was abolished by 4AP, although conduction along the internode persisted at 1.1 m s^{-1}.

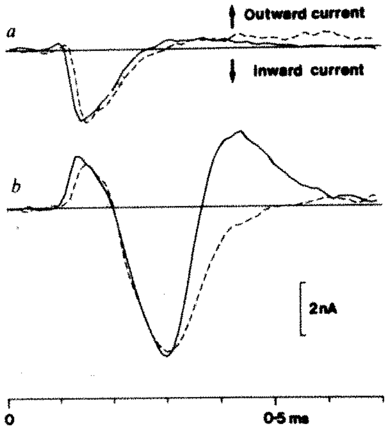

Fig. 3 Effects of 4AP on membrane currents of two consecutive nodes 1 mm apart in single dorsal root fibre exposed to diphtheria toxin. Each trace obtained by subtracting averaged longitudinal currents ($n = 64$) recorded 200 µm apart, so that points below the baseline denote inward current. Solid lines, controls; dashed lines, following application of 5 mM 4AP.

We have observed the abolition of a late phase of outward current in two other continuously conducting internodes treated with 4AP. We have also observed a similar effect at individual nodes showing signs of paranodal demyelination after diphtheria toxin injection. Of 30 nodes examined as in Fig. 3, we have found a prolongation of net inward current only at those eight nodes which took much longer than normal to be excited. These results strongly suggest that the axon membrane exposed by demyelination has different excitability properties from those of normal nodes, with a much higher proportion of potassium channels. This interpretation must remain speculative without voltage-clamp data, but receives some support from a recent voltage-clamp study of single rat nodes: potassium currents, normally weak compared with amphibian nodes, were increased in rats made diabetic with alloxan[4]. Whatever the explanation, the extranodal membrane more closely resembles, in its response to 4AP, the membrane of unmyelinated fibres, than that of normal nodes.

If 4AP prolongs action potential at demyelinated, but not at normal nodes, it might be expected to aid conduction through a diffuse demyelinating lesion, although it could not help transmission from the last normal node to the first affected one. We have tested the effects of 4AP on the blocking temperatures of 16 demyelinated single fibres, using the method previously described for scorpion venom[1]. The blocking temperature was raised (that is, transmission was improved) in 12 and lowered in only two cases, although in four cases the responses did not follow a simple time course. The effects were not as striking as those of scorpion venom[1], which acts by inhibiting sodium inactivation, but in view of the limited prolongation of the action potential that could occur with a blocking agent for potassium channels they were encouraging. One reason for testing 4AP was that it has already been used clinically and can be tolerated in patients at a concentration sufficient to enhance transmitter release from unmyelinated motor nerve terminals[8,9]. There are grounds for hoping that potassium channel blockers such as 4AP will also prove to be of value in the symptomatic treatment of multiple sclerosis and other demyelinating disease, through their specific action at those sites in myelinated fibres that most resemble their unmyelinated terminals.

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Abrogation of genetically controlled resistance of mice to *Treponema pallidum* by irradiation

John R. Klein, Andrew A. Monjan, Paul H. Hardy Jr & Gerald A. Cole

Departments of Epidemiology and Microbiology, The Johns Hopkins University Schools of Hygiene and Public Health, and Medicine, and the World Health Organisation Collaborating Center for Reference and Research in Treponematoses, Baltimore, Maryland 21205

Infection with *Treponema pallidum*, the causative agent of human syphilis, gives rise to a complex immune response involving both humoral and cellular components. The exact nature of this response and how it relates to the disease process is a matter of considerable speculation[1,2]. In recent years, studies have been directed towards defining the role of cell-mediated immunity (CMI) in syphilis[2]. These have been conducted mainly *in vitro* because the general unavailability of inbred rabbits, the principal animals for experimental syphilis research, has limited the application of *in vivo* procedures. A prime deterrent to using mice for the study of syphilis has been their failure to exhibit pathology, even in the face of a persistent infection[3,4]. We report here that on intradermal (i.d.) infection, transient primary lesions, characteristic of those seen in naturally acquired human syphilis, can be produced regularly in some strains of mice but not others, indicating a genetic basis for host susceptibility. Strains of mice which normally fail to develop lesions, do so after exposure to ionising radiation. Evidence is presented for a multiple role of the immune response during local infection.

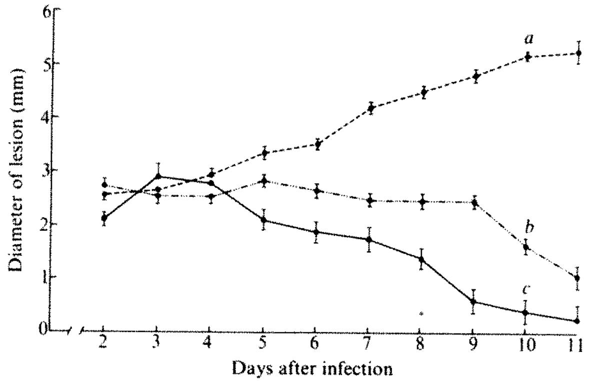

Fig. 1 Progression of lesion size in infected C3H/He mice. Each point represents the mean value ± s.e.m. of 15 normal mice (*c*), 10 irradiated mice reconstituted with non-immune cells as described for Table 2 (*b*), and 15 irradiated mice (*a*). The kinetics of lesion development seen in irradiated BALB/c and Swiss mice were the same as shown for C3H/He mice (*a*). Inoculation procedures consisted of i.d. injection into an area of the backs of anaesthetised mice from which hair had been removed by clipping and the application of a depilatory cream. Each animal received 0.01 ml of a suspension of *T. pallidum*, 6×10^8 per ml, derived from an orchitic rabbit preparation as previously described[12], and an equal volume of the same suspension rendered non-infectious by aerobic storage for 4 d at 4 °C. All mice were maintained at 16–18 °C, temperatures which are known to promote cutaneous pathology in other *T. pallidum*-infected animals[8]. Inocula of $>10^4$ organisms were required to elicit a cutaneous lesion.

Mice infected i.d. and housed at 16–18 °C develop prominent lesions at the site of inoculation within 48 h, appearing as well demarcated, indurated papules which increase in size until day 4 of infection, and then gradually recede (Fig. 1). Depending on mouse strain, 40–60% of lesions become necrotic and form an overlying crust of dry inflammatory exudate from days 4 to 9 of infection. Histologically, these lesions are characteristic of the pathologic changes of primary syphilis. Beginning on day 2 of infection, there is an accumulation of mononuclear cells consisting of macrophages, lymphocytes and, subsequently, plasma cells throughout the epidermis and dermal collagen and fat. About one half of the lesions progress to ulceration or display a marked thinning of the epidermis beneath an accumulation of dense nuclear debris and inflammatory exudate, persisting until

Table 1 Frequency of lesions in *T. pallidum*-infected normal and irradiated mice

Strain	H-2 haplotype	No. mice with lesions/ no. tested (%) Non-irradiated	Irradiated
Swiss	—	3/40 (7.5)	40/40 (100)
C3H/He	k	40/50 (80)	43/43 (100)
DBA/2	d	14/35 (40)	NT
C57BL/6	b	20/54 (37)	NT
BALB/c	d	8/40 (20)	35/35 (100)
AKR	k	2/10 (20)	NT
(C3H/He × BALB/c)F$_1$	kd	10/14 (71)	NT

Lesions were scored at day 4 of infection, the time of maximum frequency in non-irradiated mice. By day 2 of infection, all irradiated mice developed lesions which persisted until death. Using a bacterial counting chamber, the concentration of treponemes in undiluted, wet-mounted preparations of serous material taken from 8-d lesions in irradiated C3H/He mice was $3.15\pm0.22\times10^9$ per ml (mean of 5 mice ± s.e.m.); similar numbers of treponemes were found in material from lesions of irradiated BALB/c and Swiss mice. By χ^2 analysis, lesion frequencies in C3H/He mice differed significantly ($P<0.005$) from all other strains except the (C3H/He × BALB/c) F$_1$ group. Frequencies in BALB/c and AKR mice were the same; differences between BALB/c and DBA/2 mice were not highly significant ($P=0.05$). NT, not tested.

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days 7 to 10 of infection. Both gross and microscopic characteristics are similar for all strains of mice tested. Sites of control inoculations with non-infectious organisms exhibit only transient, colourless blemishes during the first 24–48 h, and never develop crusted surfaces. Histological changes are minimal, consisting of occasional mild supperative inflammation which resolves within 24–48 h of inoculation.

As shown in Table 1, there is marked variation in the susceptibility of mice to lesion development which is least in outbred animals, a significant point as most published studies of murine syphilis have involved such animals[3,5]. Inbred strains display a spectrum of susceptibility with C3H/He mice developing lesions most consistently. The high lesion frequency seen in (C3H/He × BALB/c)F_1 mice may suggest that the factor(s) responsible for the clinical manifestation of infection are under genetic control, although more detailed studies along these lines will be required to define the exact nature of this relationship. Susceptibility seems not to be influenced by H-2 haplotype, a situation also applying for certain other bacterial infections of mice[6,7]. Consistent with the phenomenon of chancre-immunity that occurs in human syphilis and experimental *T. pallidum* infections of other animals[8], mice which develop primary lesions become completely resistant to i.d. treponemal challenge by day 20 of infection.

The importance of an intact immune system in the outcome of local infection was dramatically illustrated by the use of radiation-induced immunosuppression. Mice were exposed to lethal doses of total body irradiation (TBI) from a ^{137}Ce source (137 rad per min), 850–1,050 rad depending on mouse strain[9]. Mice, infected 24 h after irradiation, developed progressively enlarging soft colourless lesions with rounded surfaces that persisted until the time of death 10–14 d post-irradiation. Lesions in all irradiated mice, including those of 'resistant' strains, contained extraordinarily large numbers of viable treponemes and never exhibited the ulcerative appearance frequently seen in immunocompetent mice (Table 1). Highly motile organisms were readily recoverable from all lesions sampled, particularly between days 6 and 11 of infection, a time when treponemes were virtually absent in lesions of non-irradiated mice. Similarly, Warthin–Starry-stained paraffin sections of lesions revealed an intense accumulation of treponemes throughout their entire area (Fig. 2).

Table 2 Effect of irradiation and immune reconstitution on cutaneous lesions in C3H/He mice

			Challenge outcome	
Recipient	TBI (950r)	Spleen cell donor	No. of mice with lesions/ no. tested	Type of lesion
Non-immune	−	−	40/50	Healed
	+	−	40/40	Unhealed
	+	Non-immune	10/10	Healed
	−	Immune	0/10	None
	+	Immune	0/10	None
Immune	−	−	0/13	None
	+	−	9/9	Unhealed

Non-immune mice were age-matched uninfected animals. Immune mice were animals infected i.d. 23–28 d previously which developed primary lesions. As measured using an indirect fluorescent antibody procedure[11], the mean anti-treponemal antibody titre of immune mice was 32 (the reciprocal of the highest serum dilution giving positive fluorescence). Healed and unhealed lesions are as described in the text for non-irradiated and irradiated mice respectively. Syngeneic spleens were teased through a 60-mesh stainless steel screen. The cells were suspended and washed twice in Hanks' balanced salt solution. Cell viability was >95% as determined by Trypan blue exclusion. Animals were reconstituted with either 8×10^7 nucleated non-immune cells given intraperitoneally, or 4×10^7 nucleated immune cells given intravenously.

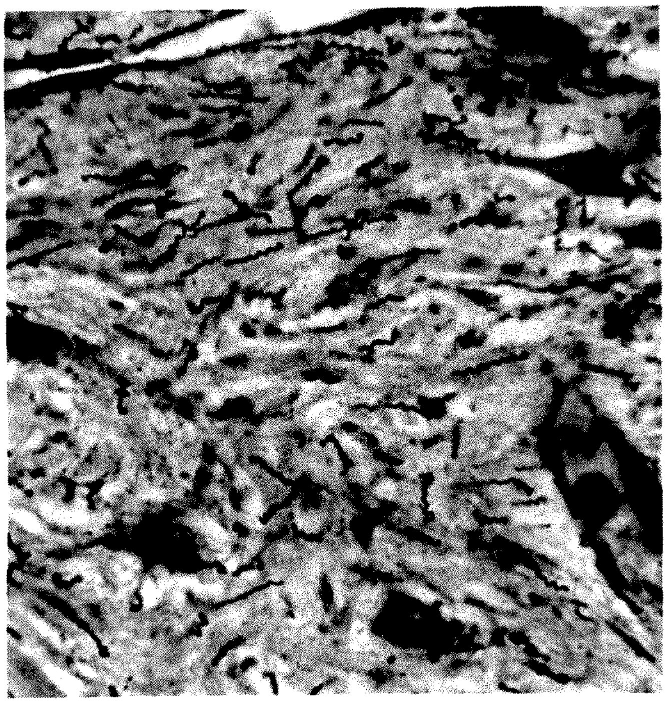

Fig. 2 Warthin–Starry-stained section of an 8-d lesion from an infected irradiated C3H/He mouse (×930). The pronounced accumulation of spirochetes in the absence of appreciable pathology is typical of lesions examined in all strains of irradiated mice (5 mice per strain). [Warthin–Starry-stained sections of lesions from non-irradiated mice reveal a gradual reduction in the number of spirochetes until day 5 of infection, after which no organisms are identifiable.]

Immunological involvement was further demonstrated by reconsituting C3H/He mice with syngeneic spleen cells within 2 h of irradiation, a procedure which not only altered the course of lesion development (Table 2, Fig. 1), but also prevented radiation death. Recipients of non-immune spleen cells, when infected 2–3 d later, developed indurated crusted lesions, similar to those seen in infected non-irradiated animals, and healing within 11 d. However, irradiated mice infected 24 h after reconstitution (data not shown) developed lesions which, like those of irradiated unreconstituted mice, progressed until day 7 of infection, except that they then became indurated and ulcerated, requiring an additional 10–12 d to heal. In these conditions, it seems that treponemes became firmly established in cutaneous tissues before immune function was restored, resulting in a biphasic lesion morphology. Collectively, these results indicate that potentiation of treponemal infection in irradiated mice is primarily the result of abrogating the immune response. As shown in Table 2, recipients of immune spleen cells, with or without preceding irradiation, are protected against any type of lesion development after i.d. challenge. However, irradiation of immune mice reestablishes their susceptibility and, when challenged, they develop large papular lesions, rich in motile treponemes, and identical to those produced in non-immune irradiated mice. Note that irradiation had no detectable effect on existing anti-treponemal antibody levels in immune mice when tested 10 d post-irradiation (Table 2).

This study points to two distinct roles for CMI during local *T. pallidum* infections. First, it undoubtedly is responsible for the characteristic pathogenesis of primary lesions as has been suggested from previous studies of treponemal infections in rabbits immunosuppressed with cyclophosphamide or cortisone[8,10]. By combining irradiation of the murine host with immune reconstitution, we have obtained direct evidence for the immune-mediated basis of these lesions. Second, the cellular component of the anti-treponemal immune response seems to be required for both the resolution of local infection and protection against subsequent i.d. challenge; its absence permits treponemes to thrive at the site of infection with only minimal tissue damage. We consider this model to have much potential application to the study of syphilis immunobiology since it should enable the identification of *T. pallidum*-specific effector cells and permit their functional activity to be defined *in vivo*. It may also serve as a new tool for evaluating both the virulence of different strains of *T. pallidum* and the immunoprophylactic value of potential non-infectious syphilis vaccines, including purified treponemal antigens. Finally, the model lends itself to further studies of host genetic factors in disease susceptibility.

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Requirement for monocytes in the spontaneous cytotoxic effects of human lymphocytes against non-lymphoid target cells

J. E. de Vries*, J. Mendelsohn & W. S. Bont*

Division of Hematology/Oncology, University of California San Diego, La Jolla, California 92093

Human lymphocytes have been shown to lyse *in vitro* a large variety of target cells derived from tumour as well as normal tissues[1,2]. The effector cells involved in this spontaneous cell-mediated cytotoxicity (SCMC) have generally been characterised as lymphocytes which lack surface membrane immunoglobulin (sIg) and lack or express only low-affinity receptors for sheep red blood cells (SRBC), but possess receptors for the Fc portion of IgG (refs 3–7). We have previously shown that monocytes may be involved in SCMC, as strong depletions of adherent cells always resulted in a reduction in SCMC[8]. In the present study, experiments were undertaken to determine the role of monocytes in SCMC. Evidence is presented that monocytes have a helper function in the lymphocyte-mediated SCMC against target cells growing in monolayer cultures, whereas effector cells in SCMC against lymphoid target cells growing in suspension cultures were lysed by lymphocytes in the absence of monocytes.

SCMC was tested in a 16-h ^{51}Cr-release assay against target cells from T-24 bladder carcinoma, NKI-1 and NKI-10 melanoma, and EK embryonic kidney cell lines, growing in monolayers, and in a 4-h ^{51}Cr-release assay on lymphoid target cells from K562 (myeloid leukaemia) and Molt-4 (T-cell leukaemia) growing in suspension cultures. All cultures and tests were carried out in Dulbecco's modified Eagle's minimal essential medium (DMEM) supplemented with antibiotics and 10% fetal calf serum (FCS). Ficoll/Hypaque-isolated mononuclear leukocytes from peripheral blood, very pure lymphocyte fractions (LF) and highly enriched monocyte fractions (MF) were used as effector cells. The LF and MF were obtained by separating the Ficoll/Hypaque-isolated mononuclear leukocytes according to size by velocity sedimentation at unit gravity in a LACS-II apparatus[9]. Both fractions are characterised in Table 1.

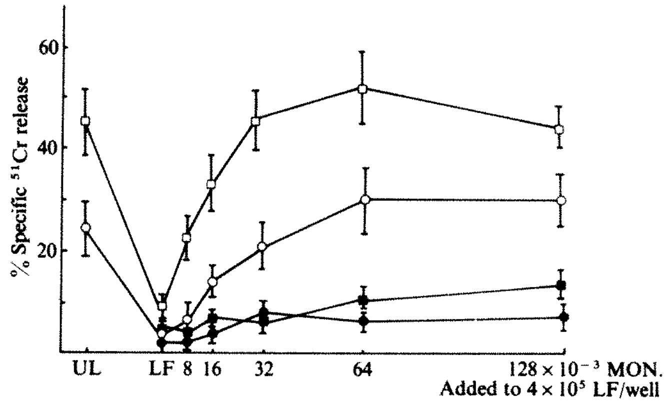

Fig. 1 Restoration of SCMC with autologous monocytes (mean ± s.e.m. of four experiments). 10^6 Monocytes were incubated for 3 h at 37°C in 1 ml culture medium containing 300 μg carrageenan under constant shaking. Before testing, the monocytes were washed three times by centrifugation. □, T-24; ○, NKI–1; ■, LF tested on T-24 after addition of carrageenan-treated autologous monocytes; ●, LF tested on NKI–1 after addition of carrageenan-treated autologous monocytes.

Compared with the corresponding unfractionated mononuclear leukocytes (UL), the LF and MF of 14 different healthy donors showed markedly reduced SCMC against the monolayer targets. In contrast, LF cells were nearly as effective as the corresponding UL in SCMC against K562 and Molt-4 target cells growing in suspension cultures, whereas the MF cells were insignificantly or weakly cytotoxic for these targets.

To investigate whether the loss of SCMC of the LF against monolayer targets could be attributed to the monocyte depletions, reconstitution experiments were carried out with autologous MF cells (Fig. 1). Addition of approximately 2% monocytes to LF cells resulted in significantly increased SCMC, whereas 8–15% monocytes were sufficient to restore the SCMC to the level achieved with the corresponding UL. Optimal induction of SCMC was observed after addition of 15–30%

Table 1 Characterisation of leukocyte fractions

	UL	LF	MF
Sedimentation velocity	—	20–36 mm h^{-1}	50–62 mm h^{-1}
% Monocytes (esterase)	23 ± 4	0.2	83 ± 4
% Monocytes (phagocytosis)	20 ± 5	<0.1	79 ± 5
% Monocytes (electronic sizing)	22 ± 4	—	81 ± 4
% Lymphocytes	77 ± 5	99.8	16 ± 4
% Lymphocytes forming SRBC rosettes	51 ± 6	56 ± 6	9 ± 3
% Lymphocytes with Fc receptors	15 ± 3	17 ± 4	10 ± 3
% Lymphocytes with C_3 receptors	18 ± 4	20 ± 3	5 ± 2
% Lymphocytes with sIg	6 ± 1	7 ± 2	3 ± 1
Granulocytes (basophils)	<0.5	0	1

Characterisation of the UL, LF and MF (mean ± s.d. of five different donors). The different criteria used to characterise the UL, LF and MF were described previously[10]. In all instances, at least 300 cells were counted. For estimating the monocyte contamination of the LF, up to 5,000 cells were screened. Of the lymphocytes present in the cell sample, 60% were recovered in the LF, whereas approximately 40% of the monocytes were obtained in the MF.

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*Present address: The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.

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Table 2 SCMC of unfractionated lymphocytes (UL), lymphocyte fraction (LF) and monocyte fraction (MF)

No. of effector cells added	T-24			NKI-1			NKI-10			EK			K562			Molt-4		
	UL	LF	MF	UL	LF	MF	UL	LF	MF	UL	LF	MF	UL	LF	MF	UL	LF	MF
4×10^5	48±7	8±3	9±3	34±6	6±3	8±3	20±4	2±1	5±2	55±9	10±2	13±3						
2×10^5	35±5	6±3	6±2	24±5	4±2	5±2	14±4	2±1	3±2	41±7	7±2	9±2	55±5	48±7	16±3	39±4	35±5	9±2
1×10^5	23±4	4±2	3±1	17±4	2±1	4±1	9±3	0	1±1	30±5	3±1	5±2	38±5	31±4	9±2	28±5	26±4	7±2
5×10^4													31±4	25±4	4±1	20±4	19±3	5±2

SCMC (mean % of specific ^{51}Cr release ± s.e.m.) of UL and corresponding LF and MF from 14 different healthy donors was tested on T-24, NKI-1 and MKI-10 target cells. Six different donors were tested on EK, K562 and Molt-4 target cells. The ^{51}Cr-release assays on monolayer targets (T-24, NKI-1, NKI-10 and EK) were carried out in Microtest II plates (Falcon, no. 3040 F). Confluent monolayers of target cells were trypsinised. Target cells 5,000 per well were plated in 0.2 ml culture medium and incubated overnight at 37°C and 5% CO_2, and then 4 μCi ^{51}Cr was added to each well. After 4–6 h incubation, the target cells were washed three times and 0.2 ml of effector cells in culture medium was added in the concentrations indicated. Effector and target cells were incubated for 16 h after which 100 μl supernatant was collected and counted. The ^{51}Cr-release assays on K562 and Molt-4 target cells (growing in suspension cultures) were carried out in U-bottomed Linbro plates (no. 76-213-05). In these, 10^4 ^{51}Cr-labelled target cells (100 μl) were mixed with the appropriate number of effector cells (in 100 μl), centrifuged for 2 min at 150*g* and incubated for 4 h at 37°C and 5% CO_2. At the end of the incubation 100 μl supernatant was collected and counted. The specific ^{51}Cr release was calculated by the following formula: % cytotoxicity = (test ^{51}Cr release − spontaneous ^{51}Cr release)/(total amount of ^{51}Cr incorporated into the targets − spontaneous ^{51}Cr release). The average spontaneous ^{51}Cr release ± s.d. was: T-24 = 18 ± 3; NKI-1 = 15 ± 3; NKI-10 = 13 ± 3; EK = 20 ± 4; K562 = 9 ± 2; Molt-4 = 12 ± 2.

monocytes. MF cells in the absence of LF cells were not cytotoxic. Intact, paraformaldehyde-killed monocytes were ineffective in inducing SCMC (not shown).

As the MF used as monocyte source for our reconstitution experiments contained 15–20% lymphocytes, it was possible that the induction of SCMC was mediated by these non-cytotoxic lymphocytes present in the MF. To evaluate this possibility, the MF cells were preincubated with carageenan, which selectively kills monocytes[11]. The carrageenan-treated monocytes caused no induction of SCMC after addition to the LF (Fig. 1), suggesting that the helper effects were mediated by monocytes and not by the contaminating lymphocytes in the MF. Lymphocytes were not affected by the carrageenan treatment, because LF cells preincubated with carrageenan showed good responses to mitogens after addition of autologous monocytes (J.E.d.V. and L. Holley, unpublished findings). Having demonstrated that SCMC on target cells growing in monolayer cultures required the presence of viable monocytes, we next tried to determine whether soluble factors produced by cultured monocytes could induce SCMC by LF cells. Figure 2 shows that supernatants of allogeneic monocyte cultures added to LF cells induced significant SCMC, although this was always weaker than the SCMC achieved with viable autologous monocytes and never exceeded 60% of the SCMC obtained with the corresponding UL. Monocytes or monocyte culture supernatants (MCS) could not be replaced by 2-mercaptoethanol (2-ME), which also had no supplementary effect on the restorative effects of MCS.

The effector cells in SCMC against non-lymphoid target cells growing in monolayers have previously been characterised as non-SRBC-rosette forming, sIg-negative, FcR^+ lymphocytes[7,13]. FcR^+ lymphocytes were also found to be the actual effector cells in monocyte-induced SCMC by LF cells, because addition of autologous monocytes to LF cells depleted in FcR^+ lymphocytes resulted in insignificant induction of SCMC (results not shown).

These results suggest that viable monocytes or soluble monocyte products are required for FcR^+-lymphocyte-mediated SCMC against non-lymphoid target cells growing in monolayer cultures. Reconstitution with very low monocyte concentrations was sufficient to induce significant SCMC. This may explain why conventional monocyte depletion procedures, because they generally result in incomplete monocyte removal, failed to demonstrate the requirement of monocytes in SCMC measured in 16-h ^{51}Cr-release assays.

The finding that LF cells were completely able to kill K562 and Molt-4 cells in the absence of monocytes (Table 2) suggests that distinct types of effector cell are involved in the killing of target cells growing in monolayers and target cells of lymphoid origin growing in suspension cultures (the former requiring monocyte help, the latter independent of it). Our findings are consistent with the results of Timonen and Saksela[12], who demonstrated that depletion of lymphocytes which were cytotoxic for fetal fibroblasts growing in monolayer cultures did not result in reduction of SCMC against lymphoma (Raji) and leukaemia (Molt-4, K562) cell lines. This indicates that different effector cell populations may be involved in SCMC against monolayer targets and lymphoid target cells growing in suspension cultures. Such a possibility is further supported by the finding that, in contrast to the effector cells active in SCMC against monolayer targets[7,13], the effectors in SCMC against K562 may possess low-affinity receptors for SRBC[6]. However, the extent to which the monocyte-independent SCMC against K562 and Molt-4 is related to the lymphoid origin and sensitivity of these cells remains unknown.

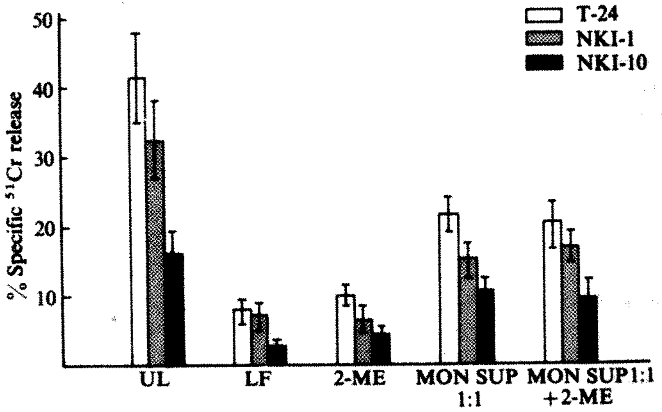

Fig. 2 Restoration of SCMC of LF cells by allogeneic monocyte culture supernatant (MCS). MCS was obtained from cultured MF cells. 4×10^5 MF cells in 0.2 ml DMEM + 10% FCS were cultures per well in Microtest II plates (Falcon, no. 3040 F). After overnight incubation (37°C, 5% CO_2) the wells were vigorously washed to remove non-adherent cells. Fresh culture medium (0.25 ml) was added and the plates were incubated for another 72 h (in other experiments 24–48 h) (37°C, 5% CO_2). The supernatants of these confluent monocyte monolayers (>96% esterase positive) were collected, centrifuged for 10 min at 2,000*g*, filter sterilised and stored at −80°C until testing. 2-ME: = 2-mercaptoethanol (5×10^{-5} M) added to LF cells from three different donors (mean ± s.e.m.); MON SUP 1:1 = MCS 1:1 diluted in culture medium added to LF cells from three different donors (mean ± s.e.m.); MON SUP 1:1 + 2-ME = MCS 1:1 diluted in culture medium and 2-ME (5×10^{-5} M) added to LF cells (mean ± s.e.m.).

As LF cells were found to be cytotoxic for Chang liver cells in an antibody-dependent cellular cytotoxicity (ADCC) system[13], we considered the possibility that the monocyte requirement for SCMC against monolayer targets could have been mediated by antibodies synthesised in the presence of, or shed by, monocytes. It has been reported that these types of antibodies may have a role in SCMC and that SCMC actually is a form of ADCC[14–17]. However, we found that goat $F(ab')_2$ fragments of purified anti-human Fab antibodies did not significantly inhibit SCMC, suggesting that antibodies are not involved in SCMC and that SCMC and ADCC must be attributed to distinct cytotoxic events[13]. It seems more likely that the induction of SCMC by monocytes is mediated by non-Ig soluble factors, as it has been demonstrated that SCMC is enhanced by purified interferon[18] and by interferon synthesised during co-cultivation of lymphocytes with tumour cells[19,20]. Therefore, the possibility must be considered that in our system the induction of SCMC is also mediated by interferon produced either directly by monocytes or indirectly in cooperation with LF cells[21,22]. These regulatory functions of monocytes in SCMC will be further investigated.

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T-lymphocyte differentiation is accompanied by increase in sialic acid content of Thy-1 antigen

Daniel Hoessli*, Claude Bron† & J. Richard L. Pink‡

*Department of Pathology, University of Geneva
†Institute of Biochemistry, University of Lausanne
‡Department of Pathology, University of Geneva, 40, Blvd de la Cluse,
1211 Geneva 4, Switzerland

Lymphocyte differentiation is accompanied by changes in the expression of various glycoproteins at the cell surface [1–4]. Whether changes in the structures of individual glycoproteins, as opposed to changes in their expression, also occur during this differentiation is not known. To answer this question, we have studied the structures of the Thy-1 glycoprotein isolated from mouse thymic (immature) and peripheral (mature) T lymphocytes. This glycoprotein was chosen for study because there is already evidence that the Thy-1 glycoprotein can carry different carbohydrate structures on the same polypeptide backbone[5] and because it is also known that peripheral T lymphocytes have more sialic acid and more sialyl-transferase activity than do thymocytes[6]. Barclay *et al.*[5] indeed showed that there were differences between the carbohydrate compositions of the Thy-1 antigen isolated from rat brain and thymus. We believe this to be the only clear example of tissue-specific differences in carbohydrate content of a given glycoprotein. In contrast to Barclay *et al.*, we have studied cells of a common differentiation pathway. Here we show, using a two-dimensional electrophoresis technique[7], that differentiation can involve the modification of carbohydrate structures of individual glycoproteins, as Thy-1 molecules on peripheral T cells carry more sialic acid than do thymic Thy-1 molecules. We also show that there is a charge difference (which does not involve sialic acid) between the two allelic forms, Thy-1.1 and Thy-1.2, of the antigen.

Thymocytes or mesenteric lymph node cells were prepared from AKR and BALB/c mice, which carry the *Thy-1.1* and *Thy-1.2* alleles, respectively. The cells were radio-iodinated and lysed in NP-40 detergent[8]. The Thy-1 antigen was precipitated from the lysates[9] using a rabbit antiserum prepared against a highly purified mouse thymus lymphocyte antigen (MTLA) which is identical with, or at least very similar to, the Thy-1 antigen present on thymocyte membranes[10,11].

Figure 1 shows that anti-MTLA specifically precipitates, from BALB/c thymocytes, a series of basic spots (*p*I above *p*H 7.5) differing in charge, with each spot showing apparent molecular weight (MW) heterogeneity in the range 25,000–30,000. Very similar spots are precipitated by conventional anti-Thy-1.2 alloantisera, or monoclonal rat anti-mouse Thy-1 reagents[12]. These results are in agreement with previous findings that the Thy-1 molecule is a glycoprotein of apparent MW about 25,000 (refs 5, 13).

Thy-1 antigens precipitated by anti-MTLA from thymocytes and lymph node cells of AKR and BALB/c mice are compared in Fig. 2. In both strains, the lymph node Thy-1 antigens are clearly more acidic than the thymus antigens. In the case of the lymph node, the spots are less heterogeneous in apparent MW, and the most acidic lymph node Thy-1 spots have a higher apparent MW than the least acidic. Similar results were obtained from splenic Thy-1 molecules.

The charge difference between thymocyte and peripheral cell Thy-1 molecules is entirely due to the presence of extra sialic acid in the peripheral lymphocyte antigens. As shown in Fig. 3, for AKR cells, the charge difference between thymocyte and lymph node cell Thy-1 molecules is abolished if the cells are treated with neuraminidase. The same is true for BALB/c cells (not shown). The thymocyte antigens do carry some sialic acid, as they become even more basic after neuraminidase treatment, but the amount of sialic acid on the Thy-1 of peripheral cells is clearly greater than that of thymocytes.

Almost all Thy-1 molecules on peripheral cells are more heavily sialylated than thymocyte Thy-1 (Figs 2, 3). The increase in sialylation is therefore probably not responsible for the antigenic difference reported to exist between the Thy-1 on thymocytes and a subpopulation (about 50%) of splenic T cells[14]. Moreover, neuraminidase-treated Thy-1 was readily precipitated by anti-MTLA, or a monoclonal anti-Thy-1 reagent (not shown).

Figure 2 also shows that AKR and BALB/c Thy-1 molecules differ in charge, with the AKR (Thy-1.1) spots being more basic than the corresponding BALB/c (Thy-1.2) spots. This inter-strain difference can also be seen in gels made from total lysates of iodinated thymocytes or peripheral cells, and is therefore not an artefact of the precipitation procedure. Also, Thy-1 molecules from AKR/Cum mice, which carry the Thy-1.2 specificity on an AKR background, behave like BALB/c Thy-1.2 mole-

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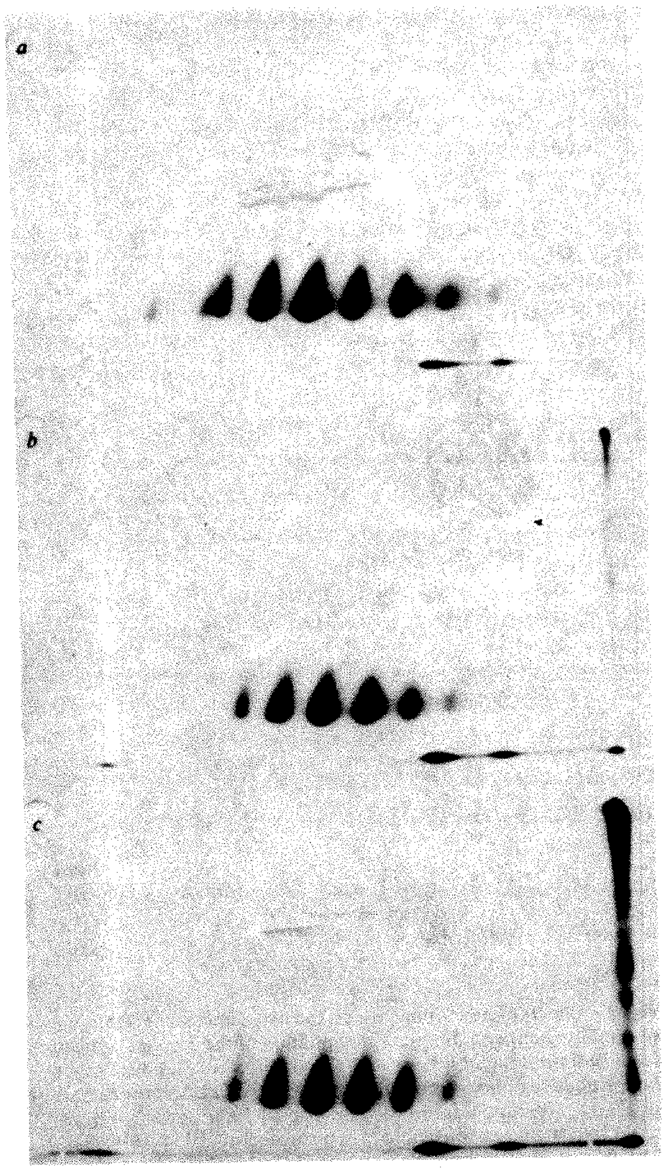

Fig. 1 Immunoprecipitation and two-dimensional gel analyses of the Thy-1.2 antigen. 50×10^6 viable BALB/c thymocytes were incubated in 1 ml of phosphate-buffered saline (PBS), 20 mM D-glucose, for 40 min at 20 °C, with 10 µg of lactoperoxidase (Sigma), 2 U of glucose oxidase (Sigma) and 1 mCi ^{125}I (NEN). After three washes in cold PBS, the labelled cells were lysed in 100 µl of PBS containing 0.5% NP-40 (v/v) and 1 mM phenylmethylsulphonyl fluoride. After a low speed centrifugation to remove nuclei, the lysate was desalted on Sephadex G-25 and the labelled macromolecular material collected in the void volume. After centrifugation at 100,000*g* for 30 min, immunoprecipitation was carried out on aliquots of lysate corresponding to 10×10^6 cells[9]. *a*, Immunoprecipitation with 5 µl anti-mouse T-lymphocyte antigen (MTLA) antiserum[10]; *b*, immunoprecipitation with 15 µl of an anti-Thy-1.2 alloantiserum (AKR anti-C3H, absorbed on AKR thymocytes) for 30 min at 20 °C, then overnight at 4 °C, followed by 25 µl of a rabbit anti-mouse Ig; *c*, immunoprecipitation with 15 µl of a rat monoclonal anti-Thy-1.2 antibody; 50 µl of a sheep anti-rat whole serum was added as a second antibody. Immunoprecipitated radioactivity was then extracted in 50 µl of O'Farrell's isoelectric focusing buffer and run in non-equilibrium *p*H gel electrophoresis (NEPHGE)[7] conditions. 10% polyacrylamide slab gels were used for the second dimension. Dried slab gels were exposed for 15–30 days to X-Omat R films (Kodak) with an intensifying screen. The basic end is at the left and the acidic end at the right of each panel, where the sample was loaded.

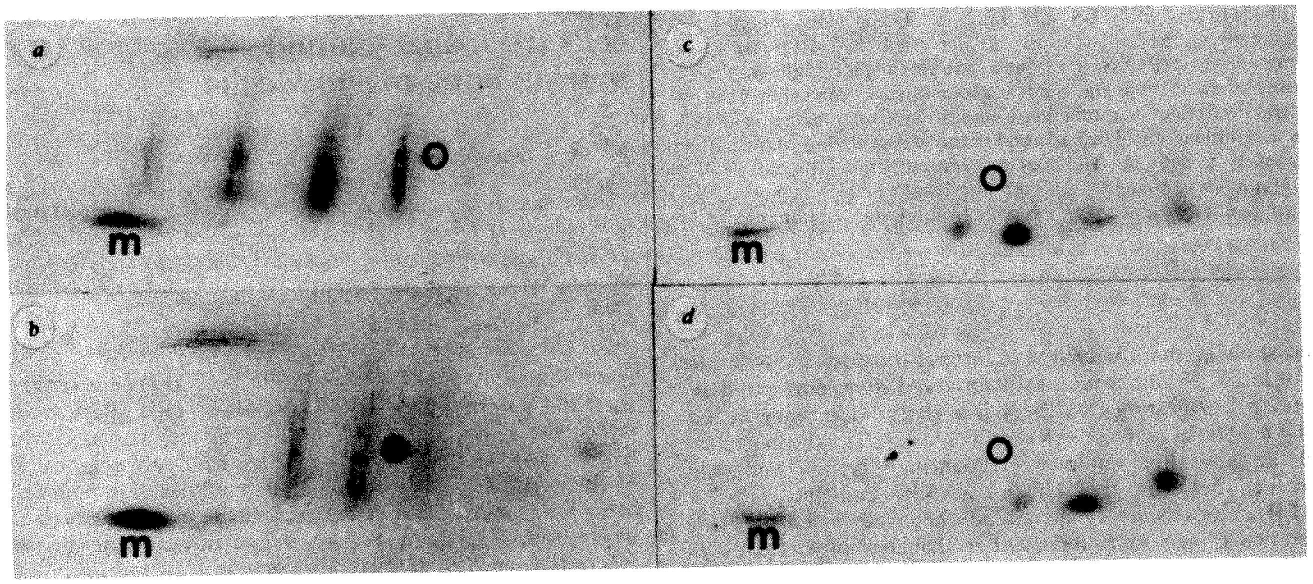

Fig. 2 Two-dimensional mapping of the Thy-1.1 and Thy-1.2 antigens. Thymocytes and mesenteric lymph node lymphocytes of AKR (Thy-1.1) origin were radio-iodinated and lysed as in Fig. 1. Lysates corresponding to 10×10^6 cells were immunoprecipitated[9] with 5 µl of anti-MTLA antiserum[10]. Immunoprecipitated Thy-1 molecules were mixed with selected isoelectric point and MW markers (m and o) labelled with ^{35}S-methionine in the isoelectric focusing buffer of O'Farrell and run in NEPHGE[7] conditions. Second-dimension gels and autoradiography were carried out as in Fig. 1. Only a portion of each autoradiogram is shown, orientated as in Fig. 1. *a*, AKR thymocytes; *b*, BALB/c thymocytes; *c*, AKR mesenteric lymph node lymphocytes; *d*, BALB/c mesenteric lymph node lymphocytes.

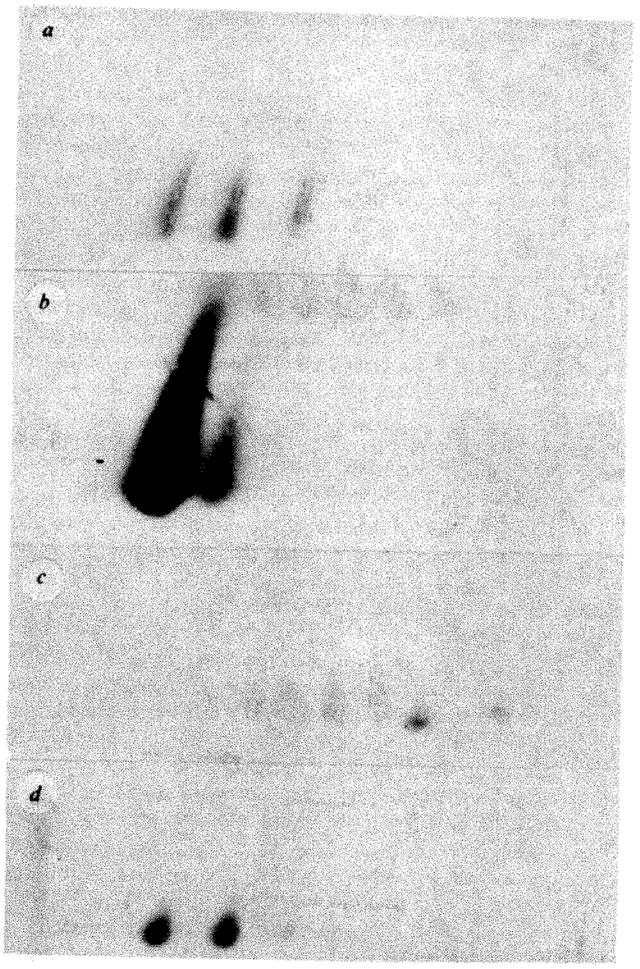

Fig. 3 Effects of neuraminidase on the two-dimensional mapping of the Thy-1 antigen. Immunoprecipitation of the Thy-1.1 antigen from radio-iodinated lysates of AKR thymocytes and mesenteric lymph node lymphocytes was done using anti-MTLA antiserum, as in Fig. 1. Neuraminidase (*Vibrio cholerae*, Behringwerke) treatment was carried out on whole cells by incubating 100×10^6 cells in 2 ml of Tris-HCl 10 mM, *p*H 6.0. NaCl 0.14 M, $CaCl_2$ 10 mM with 0.1 IU of enzyme per ml for 60 min at 37 °C. Treated cells were washed twice with PBS and radio-iodinated. Neuraminidase treatment in these conditions did not result in a decrease in lymphocyte viability. Second-dimension gels and autoradiography were carried out as in Fig. 1. Portions of autoradiograms shown are aligned on minor neuraminidase-insensitive contaminants in the immunoprecipitate. The basic end of the *p*H gradient is to the left of each panel. *a*, Thymocytes, untreated; *b*, thymocytes, neuraminidase treated; *c*, mesenteric lymph node lymphocytes, untreated; *d*, mesenteric lymph node lymphocytes, neuraminidase treated.

cules, showing that the charge difference is associated with the *Thy-1* locus. The difference is still observed after neuraminidase treatment, suggesting that there is a charge difference at the amino acid level between AKR and BALB/c Thy-1 molecules. Such an alteration could be responsible for the antigenic difference between the Thy-1.1 (AKR) and the Thy-1.2 (BALB/c) molecules. It has already been suggested that this difference is due to protein rather than carbohydrate polymorphism[5], but a role for carbohydrate in Thy-1 antigenicity[15] is not excluded.

It is not clear whether changes in the carbohydrate structure of given glycoproteins commonly accompany cellular differentiation. At least one other group of T-cell high MW glycoproteins also undergoes increased sialylation during T-lymphocyte maturation[16], but not every lymphocyte glycoprotein is modified when the cell leaves the thymus (D.H., P. Vassalli and J.R.L.P., in preparation). The increased sialylation of the Thy-1 and high MW glycoproteins cannot by itself account for the increased sialic acid content of peripheral T cells, both because peripheral lymphocytes carry less Thy-1 than do thymocytes[3], and because the number of Thy-1 and high MW glycoprotein molecules per peripheral cell ($<10^6$)[3] cannot completely account for the difference in sialic acid content between the two cell types ($>2 \times 10^7$ molecules per cell)[6].

The overall increase in sialic acid content of peripheral T cells may be functionally significant. Woodruff and Gesner[17] have shown that neuraminidase-treated peripheral lymphocytes do not home to lymphoid organs when injected intravenously into syngeneic hosts, but are quickly trapped in the liver. Moreover, normal, poorly sialylated thymocytes do not home efficiently to peripheral lymphoid organs[18]. Changes in the structure of glycoprotein carbohydrates may be as important for cellular function as the quantitative changes which occur in the amounts of cell-surface glycoproteins during lymphocyte maturation[1–4].

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Autoreactive natural killer-like cells from agar-cloned murine bone marrow cells

M. H. Claësson*† & L. Olsson‡

† University of Copenhagen, Panum Institute, Anatomy Department A, 3, Blegdamsvej, DK-2200 Copenhagen

‡ Department of Tumor Virus Research, Institute of Medical Microbiology, 30, Juliane Mariesvej, DK-2100, Copenhagen, Denmark

Natural surveillance against malignant cells has been ascribed to so-called natural killer (NK) cells. These cells attack tumour cells without prior antigen stimulation and may also be cytotoxic to non-malignant cells[1,2]. NK cells are routinely demonstrated by their cytotoxic activity against various types of tumour cell *in vitro*, but elucidation of the characteristics of the cytotoxic cells has hitherto been hampered by the lack of techniques by which NK cells can be isolated. Here we demonstrate murine NK-like activity from bone marrow cells cloned in semi-solid agar medium for granulocyte–macrophage colony formation. Data obtained indicate that such procedures considerably improve the possibility of obtaining quite pure NK cell populations by which the NK cell phenomenon may be studied in more detail.

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* To whom reprint requests should be addressed.

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Dose–response data for the cytotoxicity of mass-collected cluster and colony cells from day 7 cultures show (Table 1) that a variety of syngeneic, allogeneic and xenogeneic tumour cells as well as fetal syngeneic fibroblasts are sensitive to cytotoxic attack in a ratio of target : attacker cell as low as 1 : 0.2 to 1 : 0.1. The human Burkitt lymphoma cell line (Raji) was resistant to killing, but all other tumour lines examined were sensitive. The most sensitive targets were the syngeneic YAC-1 tumour cell line and the malignant cell line from metastatic foci of the allogeneic Lewis lung tumour model described recently[3].

Table 1 Cytotoxicty of mass-collected cluster and colony cells against syngeneic fibroblasts and syngeneic, allogeneic and xenogeneic tumour cell lines

Target cells	Target: attacker cell ratio 1:2	1:0.66	1:0.20	1:0.10	1:0.04
Fetal fibroblasts	48%*	39%	38%	20%	NS
YAC-1 (BALB/c)	40%	41%	41%	37%	27%
NS-1 (BALB/c)	27%	20%	21%	26%	NS
BW 5147 (AKR)	16%	19%	18%	NS	NS
Lewis lung (C57B1) metastatic foci	18%	17%	14%	NS	NS
Lewis lung primary tumour	57%	39%	41%	15%	NS
Raji (human) (Burkitt lymphoma)	NS	NS	NS	NS	NS
Molt-3 (human) (T-leukaemia)	21%	21%	24%	NS	NS

The cytotoxic properties of cluster and colony cells, that is, aggregates composed of less or more than 50 cells, from 7-d bone marrow cultures with 2×10^5 cells per ml agar medium[9]. Mice were 2–3 months old BALB/c. Colony-stimulating factor (GM-CSF) was a fivefold concentrated supernatant from concanavalin A stimulated sileen cells[10]used in a concentration of 1.25%. Cluster and colony cells were mass collected by mechanical disruption of the agar in serum-free RPMI-1640 medium. The agar was removed by centrifugation over fetal calf serum (FCS) at 1,000*g* and the pelleting cells were washed twice and counted for viability before assay. When individual colonies were assayed they were transferred from the agar to 0.4 ml RPMI-1640 medium by a Pasteur pipette and dispersed by pipetting. The cytotoxic properties of the collected cells were tested in a 4-h ^{51}Cr-release assay[11]. The malignant cell lines and non-malignant embryonic BALB/c fibroblasts in their second passage were labelled with ^{51}Cr and used as target cells. Briefly, fivefold replicate cultures of 10^4 target cells in 0.1 ml RPMI-1640 medium labelled with ^{51}Cr were added to wells of 96-well microplates followed by addition of 0.1 ml attacker cells in appropriate number according to the target: attacker cell ratio. When individual colonies were assayed cultures were run in duplicates and the target cell number was 5,000. The supernatant of each well was collected 4 h later and the ^{51}Cr content determined. Total release was determined by addition of distilled water to some wells. Spontaneous release was determined by release in wells containing only ^{51}Cr-labelled target cells. Spontaneous release was 10–15% of total release. s.e. values were about 5–10%. The specific release was calculated as: [(counts in experiment − counts in sponetaneous release)/(total release − spontaneous release)] × 100. In fivefold replicate cultures specific release more than 10% was significant at $P < 0.01$ level. In duplicate cultures (assay of single colonies) specific release was considered significant when equal to or more than 20%.

* Specific ^{51}Cr release, mean of fivefold replicate cultures.

Table 2 shows the kinetics of appearance of cytotoxic activity in relation to duration of culture. The cytotoxic activity was highest on days 6–7 of culture and no autoreactivity was detected on days 4 and 12.

Mass-collected cluster and colony cells obtained on day 7 were separated according to the presence or absence of Fc receptor and the cytotoxicity tested (Table 3). Only the Fc receptor-negative cell population was cytotoxic. Table 3 also shows that mature granulocyte colony cells collected from day 8 cultures were without cytotoxic effect. Similarly, bone marrow cells cultured in liquid medium in the presence of concanavalin A and tested for cytotoxicity after 4–11 d of culture showed no cytotoxic activity. Both mass-collected mouse B-lymphocyte colony cells and human T-lymphocyte colony cells were without cytotoxic activity. Table 3 includes results obtained with normal and activated adherent mouse peritoneal cells. None of these cell categories was cytotoxic. Finally, Table 3 shows that the cytotoxic activity of mass-collected cluster and colony cells from bone marrow cultures was lost by freeze-thawing the attacker cells before assay.

Of 115 single colonies from day 6–7 bone marrow cultures assayed individually (Table 1 legend) for cytotoxicity against both YAC-1 tumour cells and fetal syngeneic fibroblasts, 16 cytotoxic colonies were detected. Of these, 14 were cytotoxic to YAC–1 tumour cells (mean specific ^{51}Cr release 31%, range 23–40%) and nine of the colonies were cytotoxic to syngeneic fibroblasts (mean specific ^{51}Cr release 26%, range 20–38%). Seven of the colonies were cytotoxic to both tumour cells and fibroblasts. Four replicate agar cultures containing 2×10^5 bone marrow cells per culture were typed at day 7. Individual colonies were transferred to microscopic slides, fixed in glutaraldehyde (2.5%) and stained with Mayer's haematoxylin followed by Giemsa staining. Of these colonies 66% were of the mononuclear–macrophage type, including the dispersed type of colonies described below, 16% of the colonies were composed mainly of polymorphs and 18% were typical mixed macrophage–granulocyte colonies (Fig. 1). The cultures contained fivefold as many clusters which were not typed.

In separate experiments, morphology and cytotoxicity of individual colonies were studied simultaneously. Half of the colony was fixed and stained as above and the other half was assayed for cytotoxicity. The colony type that most often showed cytotoxic activity appeared in the agar as very dispersed aggregates composed of uniform, small cells (Fig. 1*a*). The cell number in these colonies at day 7 of culture was 500–1,000. Differential counts of five cytotoxic colonies of the dispersed type revealed that 64 ± 18% of the colony cells were small, mononuclear cells with a diameter below 10 μm. The cytoplasm of these cells was pale and contained a few small nonspecific granules but no phagocytosed agar. In contrast, the few mature macrophages (6 ± 5%) present in these cytotoxic colonies all contained large spheres of phagocytosed agar in their cytoplasm. In all the cytotoxic dispersed colonies a considerable number of polymorphs were present. The dispersed type of cytotoxic colonies constituted 3–4% of all colonies at day 7 of culture. More rarely, cytotoxic cells were present in colonies which appeared in the agar as composed of macrophages only or macrophage–granulocyte mixtures (compare Fig. 1*b*, *c*). However, in these colonies the small mononuclear cell type described above was always present in significant numbers.

Table 2 Cytotoxicity of mass-collected cluster and colony cells after different periods of culture against YAC-1 tumour cells and syngeneic embryonic fibroblasts (Fib)

Days of culture	Target: attacker cell ratio 1:1		1:0.33		1:0.10		1:0.05		1:0.02	
	YAC-1	Fib	YAC	Fib	YAC	Fib	YAC	Fib	YAC	Fib
4	40%*	NS	20%	NS	NS	NS	NS	NS	NS	NS
5	47%	32%	33%	NS	14%	NS	NS	NS	NS	NS
6	60%	52%	12%	24%	NS	NS	NS	NS	NS	NS
7	58%	33%	35%	34%	26%	NS	NS	NS	NS	NS
9	29%	14%	26%	14%	15%	NS	NS	NS	NS	NS
12	16%	NS	15%	NS	NS	NS	NS	NS	NS	NS

For explanations of technique see Table 1 legend. NS, non-significant ^{51}Cr release.

* Specific ^{51}Cr release, mean of quintiplicate cultures.

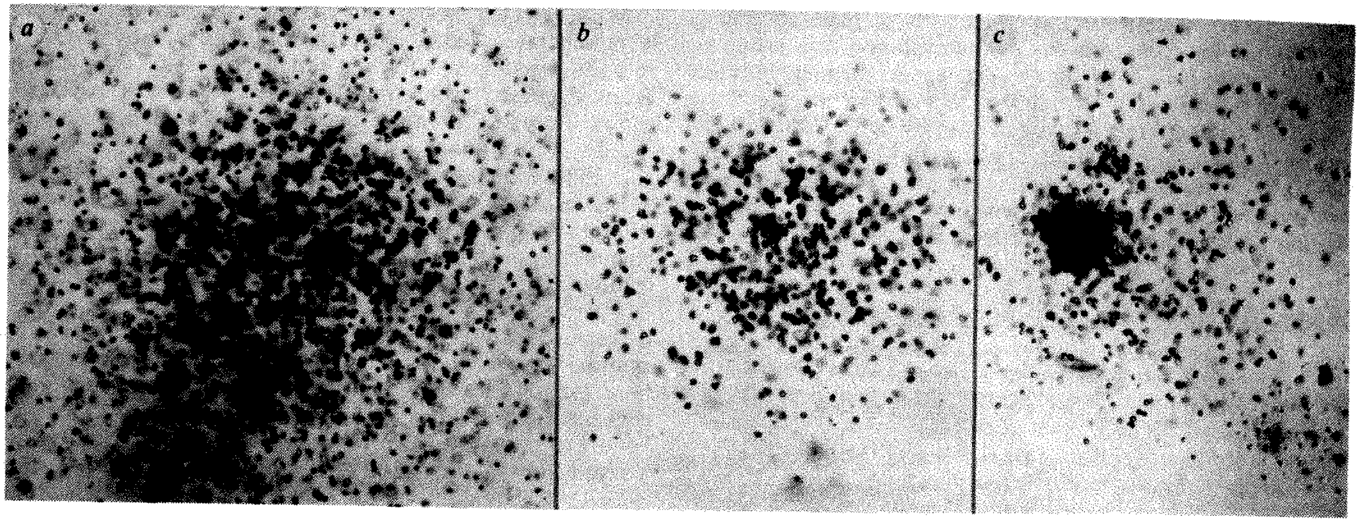

Fig. 1 *a*, A Colony with cytotoxic activity photographed in the agar medium. Note the uniform size of the very dispersed colony cells. *b*, A typical macrophage colony. *c*, A mixed macrophage–granulocyte colony. ×15.

The present results clearly demonstrate that cells with an autocytotoxic effect and with NK-like activity can be grown on a clonal basis from bone marrow cells stimulated by GM-CSF in agar medium. Clones with cytotoxic activity contain in addition to typical polymorphs and macrophages a substantial number of small mononuclear cells. It is known that both macrophages and polymorphs derived from GM colonies possess the Fc receptor[4], whereas the cytotoxic cell in the present study was Fc receptor negative. Moreover, cytotoxicity was not detected in mature macrophages or polymorphs. Taken together, our results suggest that the cytotoxic cell should be sought among the small mononuclear cells most frequently present in the cytotoxic colonies of the undifferentiated, dispersed type.

Previous studies have shown that mouse NK cells are non-adherent, non-phagocytic cells lacking the Fc receptor for IgG (ref. 5). The NK cell also lacks surface receptors typical for B and T lymphocytes[6], but has been suggested to belong to a pre-T-lymphoid subpopulation[1]. The present culture system is presumed only to permit proliferation of non-lymphoid precursor cells, that is, GM-colony forming cells[7]. However, it remains to be proven that non-B, non-T-lymphoid cells cannot proliferate and differentiate to a certain degree (without gaining receptors typical of mature lymphocytes) in the present agar culture system. Therefore, we cannot exclude the possibility that the cells with NK-like activity might be subsets of lymphoid cell precursors.

Table 3 Cytotoxicity of different cell populations against YAC-1 tumour cells and syngeneic embryonic fibroblasts (Fib)

Attacker cell	Target : attacker* cell ratio	Target cells YAC-1	Target cells Fib
Unseparated GM-cluster and colony cells	1:0.4	41%$^+$	39%
Fc$^+$ fraction†	1:0.4	NS	NS
Fc$^-$ fraction†	1:0.2	25%	22%
Freeze-thawed cluster and colony cells	1:0.4	NS	NS
Mature granulocytic colony cells‡	1:1	NS	NS
Bone marrow cells in liquid culture + Con A, days 4–11 of culture§	1:10	NS	NS
Mouse B-lymphocyte colony cells[13]	1:1	NS	NS
Human T-lymphocyte colony cells[14]	1:1	NS	NS
Peritoneal adherent cells			
normal	1:2.5	NS	NS
activated‖	1:7	NS	NS

* Highest number of attacker cells assayed.

† Prepared according to ref. 12. Separated on Isopaque–Ficoll at 1,700*g*.

‡ Day 8 GM agar culture. Morphology confirmed microscopically.

§ Bone marrow cells (2×10^6 per ml RPMI-1640 medium + 10% FCS + 5 μg concanavalin (Con A)).

‖ Mice injected intraperitoneally with thioglycolate 4 d before peritoneal washing and cell collection.

$^+$ Specific release, mean of fivefold replicate cultures.

We have previously shown that activated autoreactive cells possess the Fc receptor[3], and recently it was shown that NK cells which had been activated *in vitro* may also be Fc receptor positive[8]. The non-activated cytotoxic cell of the present study was Fc receptor negative. It is therefore possible that NK cells and cytotoxic autoreactive cells not only belong to the same subset of cytotoxic cells but also represent different differential steps eventually within the same clone of cytotoxic cells, as seven out of nine autoreactive colonies detected in the present study also showed cytotoxicity against tumour cells.

In conclusion, it is possible to detect cytotoxic clones by their morphology in agar and it should thus be possible by this technique to obtain highly purified preparations of the cells with both cytotoxic autoreactivity and NK-like activity. This will improve the possibility for a complete phenotypic characterisation of the effector cells and their natural biological function.

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Direct cloning and extended culture of antigen-specific MHC-restricted, proliferating T lymphocytes

Benjamin Sredni, Harley Y. Tse & Ronald H. Schwartz

Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

The development of antigen-specific, T-cell lines has been a subject of intensive investigation by many laboratories interested in the problems of the nature of the T-cell receptor and the mechanism of major histocompatibility complex (MHC) restriction. We describe here a modification of the soft agar cloning technique which has allowed us to rapidly generate and expand proliferating T-lymphocyte clones directly from murine lymph nodes. Using this technique, we demonstrate that such T-cell clones are antigen specific and require I–A compatible antigen-presenting cells for stimulation.

Analysis of the B-cell repertoire of antigen-specific receptors has been greatly facilitated by the availability of monoclonal antibodies from both myeloma tumours[1] and hybridomas[2]. In contrast, little is known about either the constant regions or the antigen-combining properties of the receptors of T lymphocytes. Some progress has been made with the use of anti-idiotypic antibodies as probes for examining individual families of T-cell receptors among whole T-cell populations[3] and through direct isolation in small quantities of hapten-binding receptors[4]. Clearly, however, analysis of the T-cell repertoire would be greatly facilitated by the ready availability of large quantities of clonal products. The early observations of Ben-Sasson *et al.*[5] indicated that specific enrichment of proliferating guinea pig T cells could be achieved using repeated stimulation with antigen-pulsed macrophages. Ruscetti *et al.*[6] then demonstrated that T lymphocytes from human peripheral blood could be maintained in long-term cultures if the growth medium was supplemented with supernatants from mitogen-stimulated lymphocytes. These studies were extended by Gillis and Smith[7] who were able to propagate both human and murine cytotoxic T lymphocytes. Recently this approach has been applied to antigen-specific T helper cells to isolate cells specific for keyhole limpet haemocyanin (KLH)[8] and sheep red blood cells[9]. Although such long-term T-lymphoblast cell lines were enriched for either alloreactive or helper T cells, they did not constitute clones of antigen-specific cells. Sredni *et al.*[10] developed a technique for cloning mitogen-stimulated T lymphocytes in soft agar and Nabholz *et al.*[11] and Fathman and Hengartner[12] used this technique to clone alloreactive murine T cells from long-term T-lymphoblast cell lines; however, the clones often lost the capacity to mediate cytotoxicity. Recently, long-term cytotoxic cell lines have been successfully cloned by limiting dilution techniques[13]. Although these results represent significant advances in the development of monoclonal populations of T lymphocytes, it has not previously been possible to directly isolate from the animal a large number of antigen-specific T-cell clones which could be reliably expanded for analysis of function and specificity.

Fig. 1 A colony of proliferating T lymphocytes derived from a single B10.A lymph node cell specific for DNP-OVA. The cells were cloned in soft agar as described in Table 1. After expansion in liquid culture (Table 2) cells from this colony were all shown to be T lymphocytes by indirect immunofluorescent staining on the fluorescence activated cell sorter using a hybridoma anti-Thy.1 reagent and a fluorscein-labelled rabbit F(ab')$_2$ anti-mouse immunoglobulin.

Primed lymph node cells from C57BL/10 congeneic mice bearing different H–2 haplotypes were cloned in soft agar as described in the legend to Table 1. A typical colony is shown in Fig. 1. The number of colonies obtained was similar for all three antigens tested—dinitrophenyl-ovalbumin (DNP-OVA), poly(Glu^{60}Ala^{30}Tyr^{10}) (GAT), and purified protein derivative of tuberculin (PPD). This clonal frequency was approximately 20 to 30 colonies per 2×10^6 cells plated or 1 to 1.5×10^{-5}. However, the colonies elicited by the different antigens were independent of one another as shown in Table 1 by the additive

Table 1 Cloning of proliferating T lymphocytes

Mouse strains	Priming antigen	Antigen *in vitro*	No. of colonies per 2×10^6 cells seeded*	Clonal frequency
B10	GAT in CFA	GAT	21 ± 3	1.1×10^{-5}
B10	GAT in CFA	PPD	32 ± 3	1.6×10^{-5}
B10	GAT in CFA	GAT+PPD	52 ± 4	2.6×10^{-5}
B10.S	GAT in CFA	GAT	2 ± 0.3	1.0×10^{6}
B10.S	GAT in CFA	PPD	31 ± 4	1.6×10^{-5}
(B10.S×B10)F$_1$	GAT in DFA	GAT	20 ± 3	1.0×10^{-5}
B10.A	DNP-OVA	DNP-OVA	24 ± 2	1.2×10^{-5}

B10 congeneic mice were primed in the hind footpads and at the base of the tail with 50 μg ml^{-1} DNP-OVA or GAT in Freund's complete adjuvant (CFA) containing H37Ra[17]. Inguinal and popliteal lymph nodes were aseptically removed 7–10 d later. Single cell suspensions were prepared and passed over nylon wool columns[14,17]. The nonadherent cells (2×10^6) were stimulated in Costar plates (no. 3524) with soluble antigen (100 μg ml^{-1}) in the presence of syngeneic irradiated (2,000 R) spleen cells (5×10^5) as a source of presenting cells[16] in a final volume of 1 ml of Click's medium[17] supplemented with 10% heat-inactivated fetal calf serum (FCS). After 3 d of culture the cells were collected, washed and cloned in soft agar in 30-mm Petri dishes[10]. The lower agar layer consisted of 2.5 ml of Click's medium supplemented with 20% FCS, 0.5% Noble agar and 20–100 μg ml^{-1} antigen. The upper layer consisted of 2×10^6 T cells in 0.85 ml of the same medium but containing 0.32% agar. The dishes were incubated at 37°C in a humidified atmosphere with 5% CO_2 for 3–5 d. During this time the majority of seeded cells died while a small number grew out into colonies, each consisting of at least 50 cells. The values presented were represent the arithmetic mean of three separate experiments.

* Mean ± s.e.m.

Table 2 Antigen specificity of cloned and expanded T cells

Stimulating antigen	Concentration (μg ml^{-1})	Incorporation of ^{3}H-thymidine (c.p.m.)* DNP-OVA clone	Incorporation of ^{3}H-thymidine (c.p.m.)* GAT clone	No. of recloned colonies/ 100 cells seeded* DNP-OVA clone	No. of recloned colonies/ 100 cells seeded* GAT clone
Medium	—	29 (±7)	16 (±3)	0	0
DNP-OVA	100	7,685 (±675)	24 (±4)	74 (±6)	1 (±1)
	300	7,714 (±838)	ND	86 (±4)	ND
OVA	100	3,774 (±438)	32 (±9)	33 (±3)	0
	300	8,381 (±929)	ND	82 (±10)	ND
TNP-OVA	100	3,887 (±739)	32 (±9)	37 (±5)	0
	300	8,765 (±1,019)	ND	85 (±9)	ND
PPD	100	34 (±7)	37 (±1)	0	0
GAT	100	39 (±3)	7,887 (±930)	0	66 (±7)
DNP-GL	100	81 (±45)	260 (±87)	0	1 (±1)
DNP-GLA	100	204 (±106)	30 (±5)	0	1 (±1)
TNP-KLH	100	44 (±8)	33 (±5)	0	0

Colonies were picked from soft agar using a stereo-microscope. They were placed in flat-bottomed, microtitre wells in Click's medium along with 1×10^4 irradiated syngeneic spleen cells, 100 μg ml^{-1} antigen and 20% (v/v) of a supernatant from Con A-stimulated spleen cells prepared as described by Rosenberg *et al.*[18]. The clones were expanded in these conditions for 2–3 weeks. Cultures were fed every 3 d by removing 50–70 μl of medium and replacing it with 100 μl of fresh medium containing antigen, irradiated spleen cells and Con A supernatant. After suitable expansion aliquots of the clones were examined for reactivity in a standard 6-d proliferation assay[14,17] using ^{3}H-thymidine incorporation (1 to 2×10^3 cells) with supplemental feeding at 3 d or by recloning (200 cells) as described in Table 1 immediately after feeding. The experiments shown were carried out with cells derived from a single clone of B10.A T cells selected for DNP-OVA reactivity or a single clone of B10 T cells selected for GAT reactivity. DNP-poly(Glu^{60}Lys^{40})(DNP-GL) and DNP-poly(Glu^{42}Lys^{28}Ala^{30}) (DNP-GLA) were gifts from Dr Charles Janeway, Department of Pathology, Yale University Medical School. The values for thymidine incorporation represent the mean of three separate experiments, the values for recloning the mean of two separate experiments. ND, not determined.

* Means ± s.e.m.

result obtained when both GAT (21 colonies alone) and PPD (32 colonies alone) were used together (52 colonies). Similar numbers of colonies were obtained from mice bearing different (MHC) haplotypes when antigens to which all strains respond, such as PPD and DNP-OVA, were used. In contrast, strains known to be low responders to particular antigens[14] yielded only a few colonies. For example, C57BL/10 (B10) is a high responder to GAT, whereas B10.S is a low responder. As shown in Table 1, the clonal frequency for GAT of B10 lymph node cells was 1.1×10^{-5} and that for B10.S was only 1×10^{-6}, yet these same two strains gave almost identical clonal frequencies for PPD. The F$_1$ hybrid between these two strains (B10.S×B10) behaved like the high responder with a clonal frequency of 1×10^{-5} (Table 1). These results correlate perfectly with the antibody[15] and T lymphocyte proliferative[14] responses to GAT found in these strains and suggest that the MHC-linked immune response (*Ir*) genes that control the magnitude of those responses also control the differences in clonal frequency.

Colonies were picked and the cells expanded in liquid culture. Cell growth required antigen, fresh antigen-presenting cells in the form of irradiated spleen cells, and growth factors from 48 h supernatants of concanavalin A (Con A)-stimulated spleen cells (Table 2 legend). More rapid expansion could be achieved if free Con A was also added; however, this was not essential. When the cells had increased in number to 10^5, individual clones were chosen to study antigen specificity. Specificity was examined by thymidine incorporation and by recloning. As shown in Table 2, a colony originally selected for responsiveness to GAT could only be restimulated by GAT. One thousand cells incorporated enough ^{3}H-thymidine to give 7,900 c.p.m. whereas the same number of cells stimulated with DNP-OVA or PPD yielded background radioactivity levels. Similarly, only GAT stimulation yielded significant numbers of colonies on recloning, 66 colonies from 100 cells plated for a clonal frequency of 6.6×10^{-1}. On the other hand a colony originally selected for responsiveness to DNP-OVA could not be stimulated by GAT or

Table 3 Control of antigen presentation by genes in the I–A subregion of the MHC

Spleen cells Strain	Spleen cells MHC*	Presence of antigen	T-lymphocyte clones (source and specificity) B10.A: specific for DNP-OVA	T-lymphocyte clones (source and specificity) B10: specific for GAT
			^{3}H-thymidine incorporation (c.p.m.)	
B10.A	kkkkkdddd	−	34 (±1)	19 (±5)
		+	16,475 (±1,379)	19 (±4)
B10	bbbbbbbbb	−	32 (±5)	27 (±5)
		+	30 (±9)	21,797 (±1,897)
B10.A(5R)	bbbkkdddd	−	32 (±2)	25 (±1)
		+	36 (±6)	17,953 (±1,661)
B10.A(4R)	kkbbbbbbb	−	35 (±3)	25 (±3)
		+	13,539 (±935)	27 (±2)
A.TL	skkkkkkkd	−	31 (±5)	15 (±4)
		+	13,943 (±403)	20 (±4)
None		−	31 (±3)	18 (±5)
		+	46 (±6)	26 (±7)

Irradiated nonimmune spleen cells from various H-2 congeneic mouse strains were tested in a 6-d proliferation assay for their ability to present antigen to 2×10^3 DNP-OVA-specific, cloned B10.A T cells or 2×10^3 GAT-specific, cloned B10 T cells. 100 μg ml^{-1} DNP-OVA was used to stimulate the B10.A clone and 100 μg ml^{-1} GAT was used to stimulate the B10 clone. The conditions were the same as those described in Table 2. The values shown are from one of four such experiments. The standard errors of the mean were calculated from triplicate determinations for each value.

*The letters refer to the haplotype source of origin of the K, I-A, I-B, I-J, I-E, I-C, S, G and D regions of the murine major histocompatibility complex (MHC).

PPD. It could be stimulated by DNP-OVA, TNP-OVA and OVA although not by the hapten on different carriers DNP-poly($Glu^{60}Lys^{40}$) and DNP-poly($Glu^{42}Lys^{28}Ala^{30}$), nor trinitrophenyl-keyhole limpet haemocyanin (TNP-KLH). The original carrier alone, OVA, was not as potent a stimulator as DNP-OVA although at high concentrations (300 $\mu g\, ml^{-1}$) it could stimulate as many colonies as the immunogen. This difference is reflected in the complete dose–response curve (data not shown) suggesting that the T-cell receptor has a slightly higher affinity for DNP-OVA than for OVA. These results indicate that the receptor is not uniquely specific for a new antigenic determinant created by the hapten-carrier conjugation but rather that the receptor recognises predominantly a carrier determinant which has been modified by the hapten.

The recloning frequency was also dependent on the concentration of OVA used. At 100 $\mu g\, ml^{-1}$, the clonal frequency was 35 per 100 cells plated (3.5×10^{-1}) whereas at 300 $\mu g\, ml^{-1}$ it reached 80–85 per 100 (8.5×10^{-1}), a level comparable to that achieved with DNP-OVA. These high recloning frequencies represent an enrichment in specific cells of 70,000-fold relative to the clonal frequency obtained with the cells freshly removed from the animal (compare Tables 1 and 2). This result strongly suggests that our single step technique has produced true T-cell clones, and that the recloning frequencies actually represent the highest attainable cloning efficiencies of the method. To prove that the colonies were really derived from a single cell, 14 of the recloned DNP-OVA colonies were expanded and tested for antigen specificity. All of them showed a pattern of responsiveness identical to that shown in Table 2 for the original DNP-OVA colony. As all the progeny are identical it is reasonable to assume that they have been derived from a single homogeneous population, that is, that the original colony was a true clone.

The requirement for fresh irradiated spleen cells to expand the clones in liquid culture suggested antigen-presenting cells were necessary to effectively stimulate the T lymphocytes. Previous studies[16] from this laboratory have demonstrated that the genes encoded in the I-A subregion of the MHC control such T lymphocyte antigen-presenting cell interactions. Table 3 shows that the same restrictions apply to the clones. A GAT-specific clone isolated from a B10 mouse and a DNP-OVA-specific clone isolated from a B10.A mouse were stimulated with their respective antigens in the presence of irradiated spleen cells from either B10 or B10.A mice or mice bearing recombinant chromosomes in the MHC. In the absence of antigen no stimulation was observed (Table 3), even if syngeneic and allogeneic spleen cells were added together or F_1 spleen cells were used (data not shown). This absence of a mixed lymphocyte reaction indicates that these two particular clones do not possess alloreceptors for B10 or B10.A MHC gene products. In the presence of antigen only spleen cells histocompatible at the I-A subregion supported a proliferative response. Thus, the DNP-OVA-specific B10.A clone was stimulated by antigen in the presence of B10.A, B10.A(4R) and A.TL spleen cells but not B10 or B10.A(5R) spleen cells. For the GAT-specific B10 clone the results were just the opposite (Table 3). These genetic results are identical to what we have previously reported for whole T-cell populations[16]. They demonstrate that the phenomenon of MHC restriction is not the result of suppression by cells other than the responding cells, but rather a failure of individual clones to respond to the antigen unless it is presented in association with the appropriate MHC products.

The method we have described provides a rapid and direct technique for the generation and expansion of antigen-specific T-lymphocyte clones. Several of these clones have been maintained in culture for 4 months without loss of specificity. The power of this method is the ability to obtain directly from the animal pure populations of cells which are present in relatively low frequency(for example, for GAT the precursor frequency = clonal frequency ÷ plating efficiency = 3 per 10^5, assuming that the recloning frequency represents the plating efficiency). Thus, any problems of *in vitro* selection inherent in the techniques of cloning long-term lymphoblast cell lines[11-13] are avoided. These clones should prove useful for the biochemical analysis of T-lymphocyte function, for the purification and characterisation of T-lymphocyte receptors and for the study of the mechanism of MHC restriction and the action of *Ir* genes.

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A monoclonal antibody to human acute lymphoblastic leukaemia antigen

Jerome Ritz, John M. Pesando, Jean Notis-McConarty, Herbert Lazarus & Stuart F. Schlossman

Division of Tumor Immunology, Sidney Farber Cancer
Institute, Harvard Medical School, Boston, Massachusetts 02115

Previous studies by Greaves[1] and others[2–6] have demonstrated the existence of an antigen associated with cells from many patients with acute lymphoblastic leukaemia (ALL) and some patients with chronic myelocytic leukaemia (CML) in blast crisis. Antisera to this common ALL antigen (CALLA) have been produced in rabbits and require extensive absorption which limits both the titre and quantity of antisera that can be generated and may result in variable specificity in different laboratories. The method for generation of specific antibody by somatic cell hybridisation introduced by Kohler and Milstein[7] has been successfully used to produce monoclonal antibodies against various normal human cell-surface proteins, including β_2 microglobulin[8], histocompatibility antigens[9], thymocyte and peripheral T-cell antigens[10–12] and Ia-like antigens[13]. The present report describes the generation and characterisation of a monoclonal antibody specific for a common ALL antigen (CALLA) previously identified by conventional heteroantisera.

Our cell fusion procedure was derived from the method developed by Kohler and Milstein[14] with several modifications described by Kennett *et al.*[15]. The MOPC-21 variant, P3/NS1/1-Ag4-1, was used as the parent myeloma cell line. Spleen cells were obtained from two BALB/c mice which had each been immunised by intraperitoneal and subcutaneous injections of 2×10^7 ALL cells emulsified in complete Freund's adjuvant. One and five weeks later, an additional 1×10^7 leukaemic cells were injected intravenously. Four days after the last injection, 2.2×10^8 spleen cells were collected and fused with 6×10^7 myeloma cells in the presence of 30% polyethylene glycol and aliquoted into seven microtitre plates (Falcon). Aminopterin was added 24 h after fusion and macroscopic hybridoma colonies became evident between 10 and 28 d in culture.

Rabbit antiserum to CALLA was generated in our laboratory[16] by immunisation with either ALL cells or the Null-ALL cell line Laz 221 (ref. 17), followed by absorption with cells from an autologous B-lymphoblastoid cell line and the leukaemic T-cell line, CEM[18]. The initial screening of hybridoma supernatants also used these cell lines, which provided large homogeneous populations of cells that had been extensively characterised with conventional heteroantisera. All cell surface characterisation was done by standard indirect immunofluorescence assay with subsequent analysis of each population by the fluorescence activated cell sorter (FACS-I) (Becton, Dickinson).

Although mice were immunised with cryopreserved ALL cells from a single patient known to be CALLA positive, hybridoma supernatants were initially screened for reactivity to the Null-ALL cell line Laz 221 to select against antibodies to clonotypic antigens. Only supernatants reactive with Laz 221 underwent further analysis with Laz 388 (a B-lymphoblastoid cell line from the same individual as Laz 221) and CEM, thereby approximating the absorptive method for production of rabbit anti-CALLA. Hybridomas reactive with Laz 221 but unreactive with Laz 388 and CEM demonstrated 'CALLA-like' specificity and were then subcultured and injected into pristane-primed BALB/c mice to produce immune ascites[15]. These ascites were then screened for reactivity against a large panel of cryopreserved leukaemic cells which had been previously phenotyped with rabbit anti-CALLA. Twenty-two different hybridoma supernatants demonstrated CALLA-like reactivity with Laz 221, Laz 388 and CEM, but only one clone had specificity for CALLA when tested with this panel of leukaemic cells. All other clones were reactive with cells from some patients with AML-, CLL-, or CALLA-negative ALL. Table 1 summarises the pattern of reactivity observed with a single hybridoma, J-5, compared with rabbit anti-CALLA. In all 70 populations, reactivity was similar to that of rabbit anti-CALLA demonstrating specificity for leukaemic cells from most patients with non-T-cell ALL and some patients with CML in blast crisis. In addition, no J-5 reactivity was detected with cells from five T-ALL cell lines (CEM, HSB-2, Laz 191, HJD-1 and HHM-1).

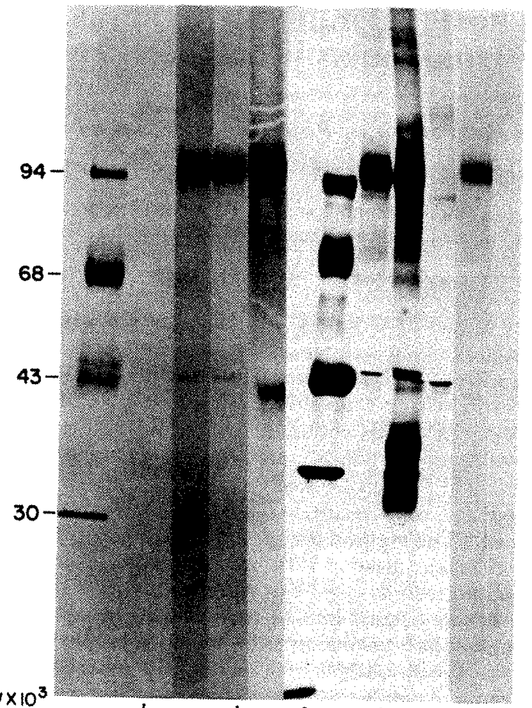

Fig. 1 Immune precipitation experiments with J-5 and rabbit anti-CALLA. *a*, ^{14}C-labelled MW standards (NEN); *b*, control monoclonal ascites with Laz 221; *c*, J-5 ascites with Laz 221; *d*, J-5 with cells from a CALLA-positive patient; *e*, J-5 with NALM-1; *f*, ^{14}C-labelled MW standards; *g*, rabbit anti-CALLA with Laz 221; *h*, rabbit anti-ALL serum absorbed only with CEM cells; *i*, normal rabbit serum with Laz 221; *j*, rabbit anti-CALLA with cells from a CALLA-positive patient. Cells were labelled with ^{35}S-methionine (NEN) for 16 h at 37 °C. Nonidet-P40-solubilised membrane extracts were applied to a lentil lectin column and bound glycoproteins eluted with 3% α-methyl-D-mannoside. Peak tubes were pooled and divided equally among samples containing antibody bound to Protein A-Sepharose (Pharmacia). After overnight incubations at 4 °C, samples were washed, heated for 5 min at 110 °C and applied to a large 7–20% gradient polyacrylamide slab gel for electrophoresis at 150 V for 18 h. Autoradiographs of dried gels were obtained by exposure for 7 d at −70 °C.

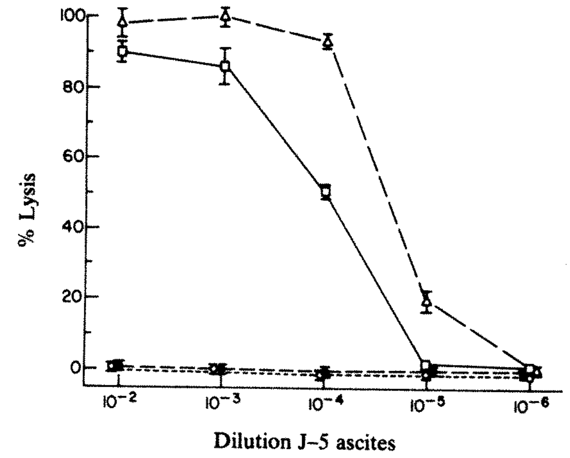

Fig. 2 Specificity of monoclonal J-5 antibody was tested using complement-mediated lysis of ^{51}Cr-labelled target cells. 4×10^3 radiolabelled target cells (20 μl) were incubated with various dilutions of J-5 ascites (20 μl) at 20 °C for 60 min followed by addition of rabbit complement (20 μl, 1:5 dilution) and further incubation at 37 °C for 60 min. Cell lysis was detected by release of ^{51}Cr into cell supernatants. All assays were done in triplicate and per cent lysis was calculated as follows: % lysis = [(experimental − spontaneous release)/(freeze-thaw − spontaneous release)] × 100. □, Laz 221; △, NALM-1; ●, Laz 388; ○, CEM.

Previous studies in several laboratories have demonstrated that heteroantisera with CALLA specificity identify a single glycoprotein with a molecular weight (MW) of 95–100,000 present on some ALL cells and the cell line NALM-1 derived from a patient with CML in blast crisis[4,19]. Figure 1 demonstrates that immune precipitates with both clone J-5 and rabbit anti-CALLA identify a single 95,000-MW glycoprotein band on cell membranes from Laz 221, NALM-1 and a CALLA-positive patient with ALL, confirming that the two antisera have similar specificity.

Specific reactivity of clone J-5 ascites was also tested by microcytotoxicity assay measuring release of ^{51}Cr from labelled target cells (Fig. 2). Greater than 50% lysis was observed with ascites dilutions of 10^{-4} on the positive cell lines Laz 221 and NALM-1, but no lysis was noted at any dilution on the negative cell lines Laz 388 and CEM. In experiments designed to test the limits of specificity and effective cytotoxicity of J-5, constant numbers of ^{51}Cr-labelled Laz 221 cells were mixed with unlabelled autologous cells (Laz 388) before addition of immune ascites (Fig. 3). Significant cytoxicity was demonstrated after a single treatment with J-5 ascites (1:1,000 dilution), when positive cells represented only 0.1% of the population mixture.

Various haematopoietic cells have also been screened for reactivity with J-5 ascites to determine whether this antibody might be identifying an antigen present on normal cells. No J-5 reactivity was demonstrated with normal peripheral blood mononuclear cells, purified T or B cells, concanavalin A-activated T cells, monocytes or spleen lymphocytes. Whereas Greaves[20] and Janossy *et al.*[21] have reported that CALLA-positive cells are present in fetal and regenerating bone marrow, we have been unable to demonstrate similar reactivity with

Table 1 Reactivity of hybridoma J-5 with leukaemic cells compared to rabbit anti-CALLA

Patients	Rabbit anti-CALLA	Hybridoma J-5
Non-T-cell ALL	21/34*	21/34
T-cell ALL	0/8	0/8
CLL	2/10†	0/10
AML	0/10	0/10
CML-blast crisis	3/5	3/5
CML-stable phase	0/3	0/3

All analyses were carried out on cryopreserved leukaemic cells obtained before the start of chemotherapy, using a standard indirect immunofluoresence assay. 10^6 cells were incubated for 30 min at 4 °C with either 0.1 ml J-5 ascites or negative control ascites (1:100 dilution to ensure antibody excess), washed twice, incubated for an additional 30 min at 4 °C with 0.1 ml fluorescein-conjugated goat anti-mouse IgG (Meloy, 1:40 dilution), and washed three times before analysis. Intensity of fluorescence with J-5 was determined for 40,000 cells in each population by the FACS-I and compared with fluorescence with negative control ascites. Any reactivity with monoclonal antibody above background was considered positive. Analysis with rabbit anti-CALLA was similar except that antisera were used at a 1:20 dilution and $F(ab')_2$ fluoresceinated goat anti-rabbit $F(ab')_2$ was used as a developing reagent.

* Each fraction represents the number of positive populations over the total number tested. Except as described below (†), leukaemic cells were either positive or negative with both rabbit anti-CALLA and hybridoma J-5. Intensity of fluorescence of positive populations, however, was not always identical and cells from three patients exhibited strong fluorescence with rabbit anti-CALLA but only weak reactivity with monoclonal J-5.

† Cells from two CLL patients were initially weakly positive with rabbit anti-CALLA but subsequent cross-absorption experiments[16] demonstrated that this reactivity was not specific for CALLA. Monoclonal J-5 antibody confirms this finding.

monoclonal J-5 antibody using both the FACS-1 and fluorescence microscopy. Eight bone marrow samples have been analysed including normal adult marrow (two volunteers) and regenerating marrow after either chemotherapy (four patients) or bone marrow transplantation (two patients). In addition, there was no detectable reactivity with J-5 ascites and 18-week fetal liver, bone marrow or thymus. This lack of reactivity with regenerating bone marrow and fetal haematopoietic tissues suggests that J-5 antibody is detecting a unique antigen which may be leukaemia specific.

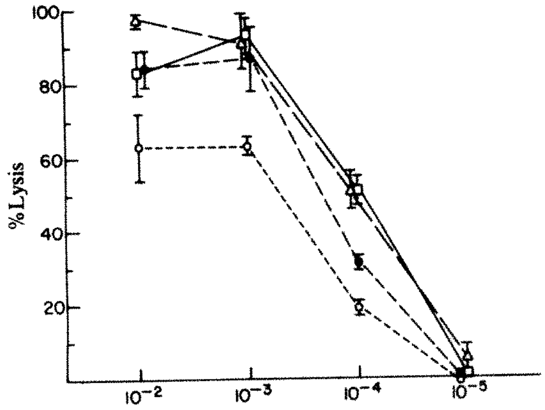

Fig. 3 Effective cytotoxicity of monoclonal J-5 was tested by mixing 10^3 ^{51}Cr-labelled Laz 221 cells with 10^4, 10^5 or 10^6 unlabelled Laz 388 cells before addition of ascites at various dilutions. Cells (100 µl) were incubated with ascites (100 µl) at 20 °C for 60 min followed by addition of rabbit complement (100 µl, 1:5 dilution) and additional incubation at 37 °C for 60 min. All assays were carried out in triplicate and per cent lysis calculated as described in Fig. 2 legend. □, 100% Laz 221; △, 10% Laz 221; ●, 1% Laz 221; ○, 0.1% Laz 221.

These studies demonstrate that J-5 monoclonal antibody will be a valuable diagnostic and therapeutic reagent. The extensive absorption required to make conventional heteroantisera specific for ALL cells and the relatively weak lytic capacity of these reagents have limited their clinical application. Development of a monoclonal antibody with CALLA specificity overcomes these obstacles and will allow clinical trials of *in vitro* and *in vivo* specific immunotherapy.

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The appearance of single-ion channels in unmodified lipid bilayer membranes at the phase transition temperature

V. F. Antonov, V. V. Petrov, A. A. Molnar, D. A. Predvoditelev & A. S. Ivanov

Research Centre, 2nd Medical Institute, Ostrovitjanova 1,
117437 Moscow, USSR

Ion-specific channels in artificial membranes have been formed by the addition of gramicidin A[1], alamethicin[2], polyene antibiotics[3] and some proteins[4] to the solution surrounding the bilayer lipid membrane. Until now there have been no reports of single-ion channels in unmodified lipid membranes. We have now studied the electrical conductance of planar lipid bilayers membranes made of synthetic distearoylphosphorylcholine (DSPC). Current fluctuations of amplitude ~1pA and duration ~1 s have been discovered at phase transition temperature, which shows that the appearance of ionic channels may be the result of lipid domain interactions. This would explain the dramatic increase in ion permeability observed in liposomes during phase transition. We suggest that these channels could conduct the transmembrane ionic current in biological membranes without the involvement of peptides and proteins.

The ionic permeability is known to change in the black lipid membrane formed from DSPC at the phase transition[5], since there is a jump in membrane conductance at 59°C, the phase transition temperature of DSPC. As the change of the integral conductance at this temperature was sharp and reversible, it was suggested that there might be a single-ion channel in the bilayer lipid membrane at this temperature.

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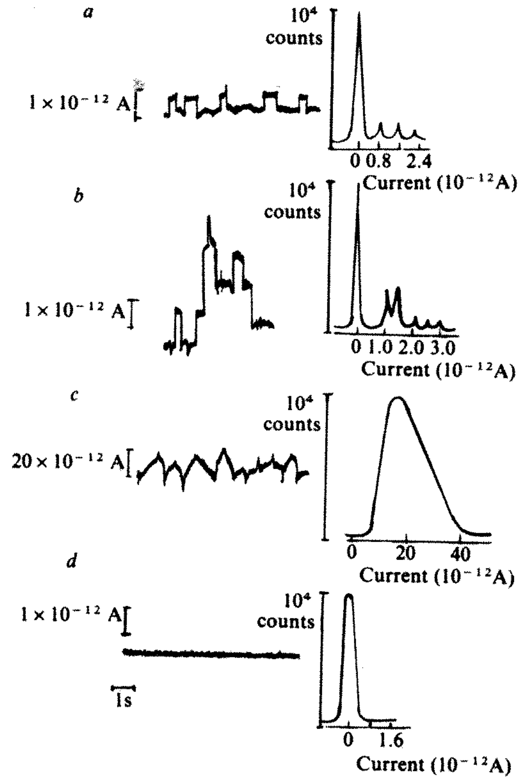

Fig. 1 Left, groups of fluctuations in the current through a lipid bilayer membrane. Right, occurrence of corresponding frequencies of the open channels, recorded by NTA-1024 multichannel analyser. The frequency was obtained by sampling the current at 200-μs intervals. [Applied potential, 100 mV.] *a*, Bilayer lipid membranes were formed from 0.2% DSPC at 59±0.2 °C. The bathing medium contained 1M KCl, 0.003M Tris-HCl *p*H 7, 4. *b*, Bilayer membranes formed from egg lecithin in *n*-decane at 22 °C. Gramicidin A 10^{-9} M was added. Egg lecithin was obtained by the method of Folch *et al.*[12]. *c*, Bilayer membranes formed from DSPC in *n*-decane at a temperature below 59 °C. *d*, Bilayer membranes formed from DSPC in *n*-decane at a temperature above 59 °C.

In an attempt to demonstrate this single-ion channel, transmembrane currents were measured in voltage-clamp conditions. The amplitude analysis of the current pulses was produced by multichannel analyser NTA-1024.

The lipid bilayer membranes were formed at holes in a Teflon 'wall' according to Mueller *et al.*[6] The temperature in the cell was kept constant by a thermostat, and the temperature around the membrane was measured by thermocouple. The DSPC used in this study was synthesised in our laboratory and contained no impurities that could be detected by thin-layer chromatography and other methods[7]. Gramicidin A (Calbiochem) was used without additional purification.

The experiments demonstrated transmembrane current fluctuation when the temperature in the cuvette was near the phase transition temperature of DSPC (Fig. 1*a*, left). The histogram reveals the discrete character of the current impulses distribution (Fig. 1*a*, right). The current fluctuation amplitude ranges discretely from 0.8 to 2.4 pA. Note that if the temperature rises above 59 °C, there are no transmembrane current fluctuations and, simultaneously, the integral membrane conductivity falls to 10^{-8} S cm^{-2}. If a noise with amplitude much higher than that of a separate current fluctuation appears, the temperature is well below the phase transition temperature (Fig. 1*c*). The integral conductance of the membrane increases sharply at this temperature. Note that these current changes are completely reversible and may repeat themselves in successive cycles of high and low temperature.

It should also be noted that the appearance of single current fluctuations (Fig. 1*a*, left) was observed in every experiment, but because of technical difficulties, it was not possible in every experiment to record sufficient fluctuations for statistical treatment (Fig. 1*a*, right) (for example, 100 single fluctuations were observed only in 10% of the experiments).

Compared to published data, it is evident that the characteristics of the current fluctuations at the phase transition are very different from those which are obtained by alamethicin[2] and EIM[4] on lipid membranes; but life times and amplitudes of the channels formed by gramicidin A in the black lipid membrane are similar (compare Fig. 1*a* and *b*).

Interestingly, the distribution of current fluctuations at the transition temperature of DSPC depends strongly on the length of time that membranes are at the fixed temperature.

The transmembrane current fluctuations reported here are evidence of the appearance of ion channels in non-modified bilayer membranes and prove that the observed ionic channels at the transition temperatures of DSPC are connected with phase transition. We assume that the ion channels appear when lipid domains interact in the membranes.

One possibility that must be considered is the involvement of impurities[10] in channel formation. This seems unlikely for the following reasons. If the channel appearance were due to impurities, we would expect a decrease in integral conductance, infrequent current fluctuation, and displacement of histogram peaks to low-amplitude values, because after repeated temperature transitions the accumulation of impurities at the phase transition should be associated with a release of some impurities into the water solvent. Therefore the content of impurities would be considerably decreased in the lipid bilayer after several repetitions of the cycle. This suggestion is based on the well known effect of zonal rectification of metals from impurities. Published data supporting this approach prove that the constant of distribution of impurities between gel and liquid crystalline phases is rather low (0.029 for dipalmitoyllecithin[8]). However, experimental data showed that neither current fluctuations nor the histogram were changed by repeated phase transitions. The integral conductance scatter is not more than 30%.

During studies of the ionic selectivity of channels we synthesised a positively charged analogue of DSPC containing a P-CH_3 group instead of P-OH group[9]. We suppose that channel walls are formed from polar groups of phospholipids. Neutral DSPC forms channels which show no ionic selectivity, whereas a positively charged analogue induces anion selectivity. This result supports the conclusion that the main phospholipids, and not impurities, are responsible for the ionic channel formation, because the synthesis schemes for the two phospholipids are the same, so the two lipid bilayers should contain identical impurities.

In bilayer membranes formed from a mixture of DSPC with 1 mol % of other synthetic lipids or egg lecithin, at the transition temperature only the DSPC-channels were recorded (data not shown). Thus lipids such as egg lecithin which constitute an impurity do not influence the formation of ionic channels at the phase transition temperature of DSPC. The suggestion impurities do not influence the formation of ionic channels is further supported by data obtained by several authors[10,11] in studies of ionic selectivity of synthetic phospholipid liposomes at the phase transition.

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Light- and GTP-regulated interaction of GTPase and other proteins with bovine photoreceptor membranes

Hermann Kühn

Institut für Neurobiologie der Kernforschungsanlage Jülich,
D-5170 Jülich, Postfach 1913, FRG

Light absorption by photoreceptor rod outer segments (ROS) leads not only to spectral and structural changes in the rhodopsin molecule[1] but also to the activation of several enzymatic activities[2–6] including GTPase[7–10]. The mechanism of this effect is not known; however, in all cases where the action spectrum of light-induced enzyme activation has been measured (see, for example, refs 4, 8), it matched the absorption spectrum of rhodopsin. This suggests that bleaching of rhodopsin is the primary step in enzyme activation, and that some light-induced changes in the molecular interaction of the enzymes with rhodopsin, the major intrinsic ROS membrane protein, should be involved. I have indeed found that light induces profound changes in the interaction of rhodopsin kinase[11], GTPase (reported here), and other proteins with the photoreceptor membrane. These changes are reversible in the dark, are strongly influenced by GTP, and are thought to be involved in the regulation of enzyme activity by light. The light-induced binding of the GTPase and its subsequent elution with GTP was used to purify this enzyme.

Bovine ROSs were purified from freshly dissected retinas by flotation and subsequent centrifugation on stepwise sucrose density gradients in 70 mM phosphate buffer[11]. Electron microscopy[12] revealed large fragments of ROS, 2–20 µm long, surrounded by a plasma membrane which looked continuous. There was no detectable contamination by other cell fragments. The ratio A_{280}/A_{500} of the ROS was 2.0–2.4, indicating good purity[13].

Gel electrophoresis of such purified ROSs (Fig. 1*a*, *b*) shows that rhodopsin is the major protein but that a number of additional polypeptides is also present, in agreement with published data[14]. Most of these additional polypeptides are soluble in the dark in hypotonic buffer (Fig. 1*d*); little protein besides rhodopsin remains with the washed membranes (Fig. 1*c*). The major four polypeptides present in dark extracts (doublet bands at molecular weights (MWs) 37,000 and 35,000 and at about 95,000) are extractable at low ionic strength but are barely extractable at moderate ionic strength (70 mM phosphate buffer[11], 100 mM Tris buffer, or 120 mM KCl Ringer's); this suggests that they are peripherally bound membrane proteins. Most of the other polypeptides can be extracted at moderate ionic strength, provided the ROS plasma membrane is disrupted, and may therefore be cytoplasmic soluble proteins or only very weakly bound membrane proteins[11].

Illumination of the ROS suspension before centrifugation causes certain polypeptides to disappear almost completely from the soluble supernatant (Fig. 1*e*; MWs 35,000, 37,000, 48,000 and 68,000, and a peptide of MW about 6,000 discernible in the front band near the bottom of the gels). These polypeptides become membrane-bound upon illumination and sediment with the bleached membranes. They are found in the membranous pellet (not shown in Fig. 1) and can be eluted from the pellet by subsequent treatments which is demonstrated, for example, in Fig. 1*o*, *p* for the polypeptides of MWs 37,000, 35,000 and 6,000. The light-induced binding to the bleached membranes is slowly reversed in the dark: If ROS suspensions are bleached but then further incubated in the dark for 70 min at 20 °C, then most of the initially bound polypeptides are found again in the soluble supernatant after centrifugation (Fig. 1*g*).

This light-induced and reversible binding has been observed previously[11] for the two polypeptides (MWs 68,000 and 48,000) which are soluble at moderate ionic strength, and the polypeptide of MW 68,000 has been identified as rhodopsin kinase. To demonstrate that the three additional polypeptides (MWs 37,000, 35,000 and 6,000) undergo light-induced changes in their interaction with the membrane, it is necessary to subject the membrane to conditions of low ionic strength. Then, light–dark differences in the binding affinity of these peripheral membrane proteins to the membrane become obvious. The binding is too weak in the dark to withstand the low ionic strength treatment; in light, however, the binding becomes much stronger, such that it resists the low ionic strength treatment for a limited time after bleaching.

Table 1 GTPase activities of the preparations shown in Fig. 1

Gel (Fig. 1)	Preparation	GTPase activity*
a	Purified ROS before extraction, 2.4 µg rhodopsin	6.0
b	As *a*, but 12 µg rhodopsin	28.8
c	Extensively washed ROS membranes, 12 µg rhodopsin	0.36
d	Dark extract, obtained from unbleached ROS (corresponding to 35 µg rhodopsin) in 5 mM Tris	41.4
e	Light extract from ROS (35 µg) bleached for 3 min, centrifuged immediately after bleaching	0.9
f	Extract from bleached ROS, centrifuged after a 2-min dark incubation period at 20 °C following bleaching	1.7
g	Extract from bleached ROS, centrifuged after a 70-min dark incubation period following bleaching	27.1
d'	Dark extract *d* assayed without addition of washed ROS	0.43
	Effect of GTP on the light-induced binding	
h	Dark extract obtained from unbleached ROS in the presence of 1 mM GTP	—
i	Light extract from ROS bleached in presence of GTP and centrifuged immediately after bleaching	—
k	Extract from ROS bleached in absence of GTP and then dark-incubated for 2 min after adding GTP	—
l	Extract from ROS bleached in absence of GTP and then dark-incubated for 70 min after adding GTP	—
	Purification of the GTPase via light-induced binding	
m	Extract obtained in a second wash of a bleached ROS pellet with 5 mM Tris	1.2
n	Further extraction of the bleached pellet by prolonged incubation with 100 mM Tris at 20 °C	0.22
o	Purified GTPase (2.8 µg protein) obtained by extracting the thoroughly washed bleached pellet with 40 µM GTP	38.0
p	Purified GTPase as *o*, but 13.8 µg protein	—

The preparations and amounts of protein used for the GTPase assays were the same as for the correspondingly lettered gels in Fig. 1. For details of the extraction procedure in darkness and light see legend to Fig. 1. The GTPase activity values given are averages of four determinations with s.d. < 5%. Assays were performed according to the method of Neufeld and Levy[20] with some modifications[8,15]. Incubation was for 2–8 min at 30 °C in continuous light. The assay mixture contained 2 µM [γ-^{32}P]GTP (except sample *o*, 3 µM ^{32}P-labelled GTP) of specific activity 2,200–3,000 c.p.m. pmol^{-1}; 20 mM Tris-HCl (*p*H 7.4); 5 mM $MgCl_2$; 1 mM dithiothreitol; and the appropriate amounts of ROS and/or extracts, in a final volume of 200 µl. To each assay of extracts (*d*–*g*, and *m*–*o*), 4.7 µg rhodopsin in the form of washed, GTPase-depleted ROS membranes was added for activation of the extracted GTPase. When GTPase-containing extracts were assayed in the absence of rhodopsin, they showed no GTPase activity (sample *d'*).

* GTPase activity is expressed as pmol P_i liberated per min at 30 °C per amount of protein seen on the gels of Fig. 1.

One of the most striking features of this light-dependent protein binding is that GTP (10^{-5}–10^{-3} M) inhibits, or reverses, the light-induced binding of the polypeptides MWs 37,000, 35,000 and 6,000 if it is present during bleaching (Fig. 1*i*) or added shortly after bleaching (Fig. 1*k*, *l*). In the dark, GTP has little influence on the extractability in hypotonic buffer (Fig. 1*h*). This solubilising effect is relatively specific for GTP—other nucleotides had only a partial effect (ATP, guanylyl imidodiphosphate) or no effect (3',5'-cyclic GMP, 5'-GMP, and others)[15]. GTP also affects the solubility of the polypeptide of MW 48,000, but in an opposite way: The light-induced binding of this polypeptide, which is normally reversible in the dark (Fig. 1*g*), becomes irreversible if GTP is present (Fig. 1*l*).

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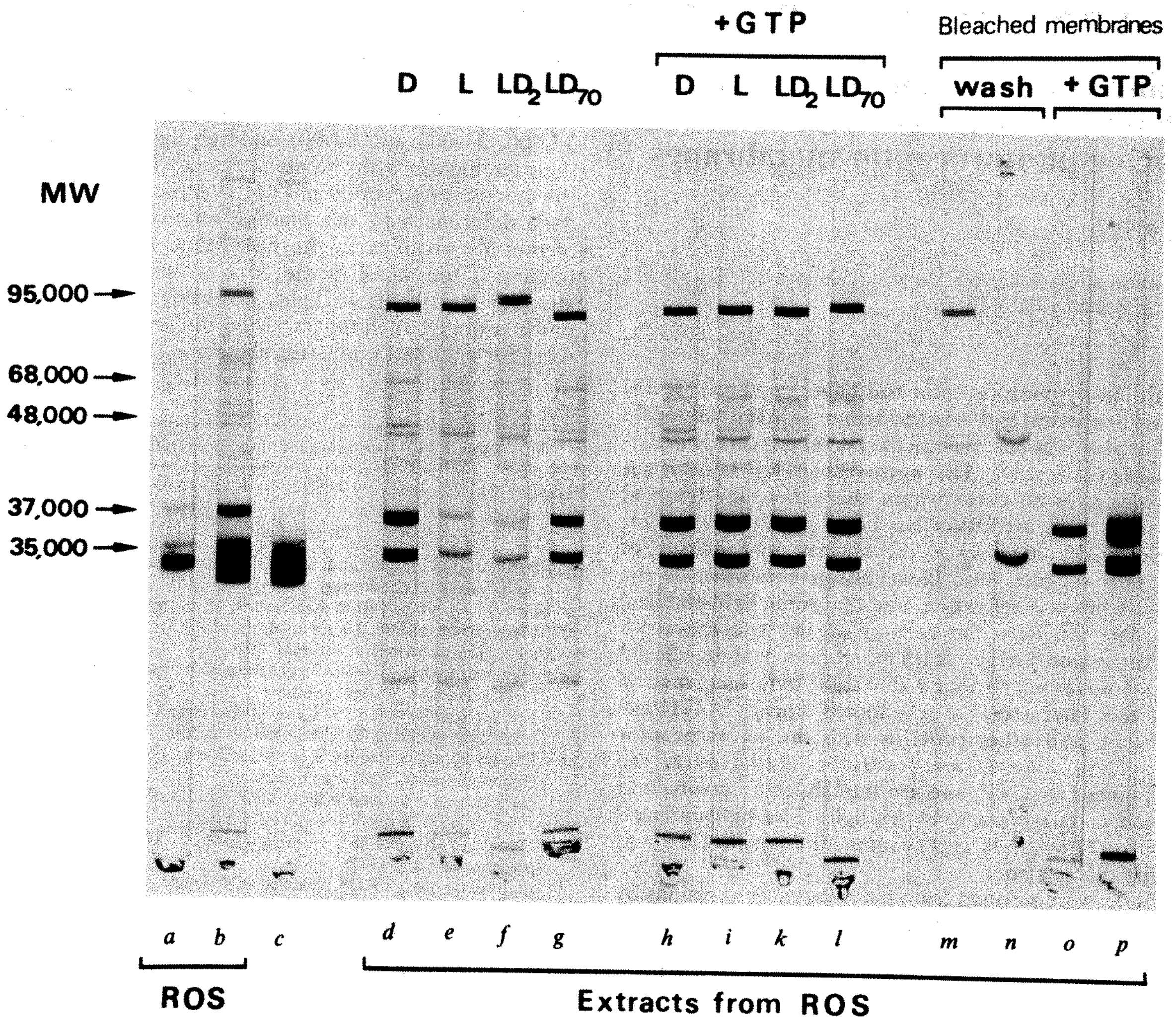

Fig. 1 Polypeptide composition of ROS and extracts, analysed by electrophoresis on discontinuous, 10% polyacrylamide gels in the presence of 0.1% SDS[17]. Gels were stained for protein with Coomassie brilliant blue. For definition of the lettered samples see Table 1, where the corresponding GTPase activities are also listed. Briefly: gels *a–c* represent ROS membranes before (*a*, *b*) and after (*c*) extraction of soluble proteins; and gels *d–p* represent soluble proteins extracted from ROS in various light/dark conditions. The extracts *d–l* were all obtained from the same ROS suspension in 5 mM Tris-HCl (*p*H 7.4): 2 mM dithiothreitol: 0.5 mM $MgCl_2$: 0.05 mM phenylmethylsulphonyl fluoride. The amount of extract on each gel (*d–l*) corresponds to 35 μg rhodopsin in the original homogenised ROS suspension. The ROSs were extracted either unbleached (*d*, *h*) or after illumination (*e–g*, *i*, *k*, *l*). The 'light' suspensions were illuminated for 3 min at 20 °C with light filtered through an orange filter ($\lambda > 540$ nm) and through a water bath, bleaching about 80% of the rhodopsin. Then both light and dark suspensions were centrifuged either immediately (*d*, *e*, *h*, *i*) or after an additional incubation period at 20 °C in the dark (*f*, *g*, *k*, *l*). (The light/dark conditions applied before centrifugation are depicted on top of the gels: D, dark extract; L, light extract; LD_2 and LD_{70}, dark incubation for 2 min or 70 min, respectively, following illumination.) The extracts *h–l* were obtained in the presence of 1 mM GTP. For details see Table 1. After the first centrifugation (10 min at 50,000*g* and 0 °C), the supernatants were carefully removed and centrifuged again for 40 min at the same speed to insure that all particulate material was sedimented. The extracts were clear and free of rhodopsin. The GTPase was purified as follows: An ROS suspension was bleached and centrifuged. The bleached pellet containing the bound GTPase was washed several times with 5 mM Tris at 0° C to remove residual soluble proteins (*m*) and was then treated several times with 100 mM Tris for 70 min at 20 °C to allow for the release of light-induced bound proteins (*n*) other than GTPase. The GTPase remains membrane-bound in 100 mM Tris. The GTPase was finally eluted by treating the pellet with 40 μM GTP in 5 mM Tris buffer (*o*, *p*). All buffers used contained 2 mM dithiothreitol. MWs of polypeptides are indicated on the left side of the gels and were estimated from gels run separately in a continuous electrophoretic system[15,18]. The most prominent band in ROS, migrating on Laemmli gels[17] below the extractable polypeptide of MW 35,000, is rhodopsin, as identified by glycoprotein staining (not shown). Polypeptides whose MWs are less than 20,000 migrate with the front on these gels[17]. This front band contains a light-dependent component which is seen in the extracts *d* and *g–l*, and in the purified GTPase (*o*, *p*) but is absent in the light extracts *e* and *f*. Analysis in a gel electrophoretic system designed to separate in the MW range of 2,000–20,000 (ref. 19) indicates a MW of about 6,000 for this light-dependent peptide. It always accompanies the GTPase and is, therefore, tentatively regarded as a subunit of the GTPase in addition to the polypeptides of MWs 37,000 and 35,000. The polypeptide of MW 68,000 carries rhodopsin kinase activity[11]. The polypeptide doublet of MW 95,000, in contrast to the other designated polypeptides, is extracted independently of light.

GTPase activities of the different fractions from Fig. 1 are presented in Table 1. Whole ROS, if assayed in the conditions described by Wheeler and Bitensky[8], exhibit GTPase activities in the same range as those reported for frog ROS in light[8]. Dark extracted and thoroughly washed ROS membranes (Fig. 1*c*) contain no GTPase activity (Table 1*c*). The extracts also show negligible GTPase activity (Table 1*d′*), unless assayed in the presence of added, GTPase-depleted bleached ROS membranes. Then the dark extracts show high GTPase activities, up to 40 times higher than the corresponding light extracts (Table 1*d*, *e*). Obviously, the GTPase is extracted from dark-adapted membranes in an inactive form and becomes active only when bleached ROS membranes are added. Extracts of bleached ROS, on the other hand, contain little GTPase because of the light-induced, strong binding of the enzyme to the membranes; but after prolonged incubation in the dark, the strongly bound GTPase is reconverted into the form where it can be extracted at low ionic strength (Table 1*g*). Comparison of GTPase activities and polypeptide composition of many extracts showed that the presence of the three light- and GTP-influenced polypeptides (MWs 37,000, 35,000 and about 6,000) always and quantitatively paralleled the presence of GTPase activity. This strongly suggests that at least one if not all of these polypeptides constitute the GTPase. This is in essential agreement with recent findings of Godchaux and Zimmerman[16] who also assign GTPase activity to the polypeptide doublet of MWs 37,000 and 35,000 (which they term 41K and 37K).

The data in Table 2 show that light changes the interaction between the GTPase and ROS membranes even in moderate ionic strength conditions where the enzyme is membrane-bound both in darkness and light (extracts D1, L1). Bleaching membranes suspended in 100 mM Tris buffer prevents the enzyme from being extracted in the dark by a subsequent treatment with 5 mM Tris (extract L2), whereas the enzyme from a similarly treated unbleached sample can be extracted in the same conditions (extract D2). It seems likely, therefore, that significant light-induced changes in the binding of the GTPase to ROS membranes occur also in physiological ionic strength conditions.

Table 2 Effect of the ionic strength on the extractability of GTPase from unbleached and bleached ROS

Treatment of ROS suspension before centrifugation	Light conditions	Extract	GTPase activity of extracts
First extraction			
ROS vigorously homogenised in 100 mM Tris and divided into two samples for dark (D)	Dark	D1	1.64
and light (L) extraction	Light	L1	1.05
Second extraction			
Pellets resulting from the first extraction	Dark	D2	20.0
resuspended in 5 mM Tris	Dark	L2	0.45

The conditions of illumination and centrifugation were as described in Fig. 1. The time elapsed between the first and second centrifugation step was 15 min during which the membranes were kept in the dark mostly at 0–4 °C. Both buffers contained 0.5 mM $MgCl_2$, 1 mM dithiothreitol, and 0.05 mM phenylmethylsulphonyl fluoride in addition to the Tris–HCl (*p*H 7.4; 100 mM or 5 mM, respectively). Neither of the extracting buffers contained GTP. The homogenisation treatment was sufficient to disrupt the ROS structure such that most of the soluble proteins were eluted in 100 mM Tris buffer, except the polypeptides of MWs 95,000 (doublet), 37,000, 35,000 and 6,000 which are membrane-bound at this ionic strength. The latter five polypeptides were found enriched in the subsequent hypotonic extract D2, whereas L2 contained only the doublet of MW 95,000 (gels not shown). GTPase activity was measured and is expressed as for Table 1.

The light-induced binding of the GTPase and its specific elution with GTP offers a unique way of purifying this enzyme. Bleached ROS are washed extensively to remove soluble proteins (Fig. 1*e*, *m*, *n*) and are then incubated with 40 μM GTP in hypotonic buffer. The resulting extract contains high GTPase activity if assayed in the presence of added bleached ROS, and the only polypeptides present are those of MWs 37,000, 35,000, and about 6,000 (Fig. 1*o*, *p*; Table 1*o*). The yield of purified GTPase is 60–70 μg of each of the two large polypeptides (MWs 37,000 and 35,000) per mg of rhodopsin; this indicates that at least 6–7 molecules of each polypeptide are present per 100 rhodopsin molecules in ROS.

The phenomenon of light-induced changes in the interaction of certain ROS proteins with the photoreceptor membrane may well reflect the mechanism by which enzymes in ROS are light-activated via bleaching of rhodopsin. To support this hypothesis, it would be important to know how rapidly the light-induced binding changes occur. Preliminary experiments[15] indicate that, even at 0 °C, both the light-induced binding and the GTP-induced release of the GTPase occurs faster than the time resolution of the centrifugation method (10–15 min). This leaves open the possibility that, at physiological temperatures, the observed changes in molecular interactions of ROS proteins may be fairly fast reactions.

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Epithelial cells in nerve-free hydra produce morphogenetic substances

H. C. Schaller*, T. Rau & H. Bode†

European Molecular Biology Laboratory, Postfach 10.2209,
6900 Heidelberg, FRG

It has been shown that hydra, consisting almost exclusively of epithelial cells, have normal morphogenetic properties. This means that they are able to reproduce asexually by budding and to regenerate head or foot with conservation of the original polarity. Such hydra have no interstitial cells and they lack nerves and nematocytes as differentiation products of the interstitial stem cell. Consequently, they are unable to catch or ingest food and would not survive in nature. However, stocks can be maintained in the laboratory, if food is injected into the gastric cavity[1–3]. The fact that nerve-free hydra would grow and regenerate normally raised some doubts as to the role of the nervous system in the control of differentiation and development in hydra. In particular, the significance of morphogenetic substances, such as the head activator[4], which is normally produced by nerve cells[5], seemed to need reassessment[6]. We now show that epithelial cells in hydra have the potential to produce head activator and other morphogenetic factors, but that this property is repressed in the presence of nerves.

In a preliminary investigation we have shown that severely nerve-depleted hydra, which consist almost exclusively of epithelial cells, contain normal concentrations of morphogenetic substances[7]. To exclude the possibility that the few remaining nerve cells overproduce these substances, we have repeated the experiments with completely nerve-free hydra. These were from two sources: the first 100 were from Richard Campbell from a

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* Present address: Max-Planck-Institute für Medizinische Forschung, Jahnstr. 29, 6900 Heidelberg, FRG.

† Permanent address: University of California, Irvine 92664.

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Table 1 Cell composition of nerve-free and normal hydra

Animal	Total cells (%)	Epithelial	Gland and mucous	Interstitial	Nematocyte lineage	Nerve
Nerve-free	14,700	13,500	660	320	210	0
	(100)	(91.8)	(4.5)	(2.2)	(1.4)	0
Normal	62,300	13,800	3,930	7,100	34,300	3,200
	(100)	(22.0)	(6.3)	(11.4)	(55.0)	(5.1)

stock originally produced by colchicine treatment and had been nerve free for more than a year[8]. The next 300–400 nerve-free hydra we produced ourselves following C. N. David's suggestion of selecting non-feeding animals from the normal culture and force-feeding them by hand. Such animals are probably the result of interstitial stem-cell depletion caused by an excessive differentiation into nerves or nematocytes or, experimentally induced, into sexual cells. Such animals were not interstitial-cell free, and they contained a few remaining nerve cells, but they produced offspring which in most cases was completely nerve free. Such nerve-free hydra were easily recognisable by their characteristic shape, with many undetached buds, swollen heads and short tentacles (Fig. 1*a*), by their inability to catch food and by their lack of reaction against tactile stimuli such as tickling the tentacles with a needle[9]. To standardise size we selected animals in which a first bud was just visible (Fig. 1*b*). Such standard animals consisted on average of 15,000 ± 2,000 cells, of which more than 90% were epithelial cells (Table 1).

Table 2 Amount of material necessary to achieve the effect corresponding to 1 biological unit (BU)

Morphogen	Nerve-free hydra	Normal hydra	Ratio
Head activator	0.009	0.075	8.3
Head inhibitor	0.009	0.033	3.7
Foot activator	0.05	0.09	1.8
Foot inhibitor	0.14	0.11	0.8

The amount of material is given in A_{280} units per ml. One standard nerve-free hydra, if homogenised in 1 ml, yielded on average 0.14 A_{280} units and one standard normal hydra 0.15 A_{280} units. One biological unit is defined as the amount of material necessary in 10 ml of medium to achieve for the head activator a 6% increase in tentacle number, a 50% increase in bud outgrowth, or a 33% acceleration in head-regeneration rate, for the head inhibitor a 50% decrease in bud outgrowth or a 33% inhibition of head-regeneration rate, for the foot activator a 50% acceleration and for the foot inhibitor a 25% inhibition of the foot-regeneration rate[10].

From these hydra we made crude extracts and assayed them directly and after purification for content of head activator, head inhibitor, foot activator and foot inhibitor[10]. We found that nerve-free hydra contain eight times more head activator, four times more head inhibitor, twice as much foot activator and a little less foot inhibitor than normal animals (Table 2). No difference was found in the head-activator concentration of nerve-free hydra originating from the two sources. From this we conclude that cell types other than the nerve cells can produce morphogenetic substances. The cell composition shown in Table 1 reflects the status of animals that have been nerve free for a few generations. The longer such animals are propagated by force-feeding, the more they lose cell types other than the epithelial cells, such that in the end they consist exclusively of epithelial cells. As Campbell's animals had reached that stage, we are sure that it is the epithelial cell which is producing morphogenetic substances. Campbell and Marcum produced nerve-free hydra by colchicine treatment[8], and Sugiyama *et al.* selected for an interstitial-cell-free mutant by sexual inbreeding[3]. The fact that our animals were asexual offspring from normal hydra, not treated with chemicals or mutagens and not the result of sexual crosses, suggests that the ability to produce morphogenetic substances is an inherent property of epithelial cells and not the result of a mutation or chemical mistreatment.

We had shown earlier that in normal animals most of the head activator (80–90%) is located in nerve cells[5]. This did not exclude the possibility that the remaining 10–20% may be present in another cell type. To obtain direct evidence on the occurrence of head activator in normal epithelial cells a procedure was devised for separating nerves from epithelial cells. Hydra were dissociated into viable cells according to the method described in Trenkner *et al.*[11]. Epithelial cells were separated from nerve cells using sedimentation velocity centrifugation in Percoll gradients. Because the two cell types differ greatly in size (average nerve cell and epithelial cell diameters are 6.0 and

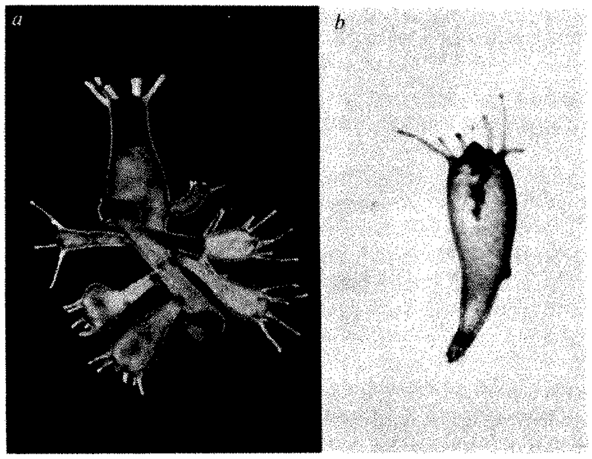

Fig. 1 Nerve-free hydra. *a*, Mother animal with several buds which need much longer to detach than normal buds. Each individual bud has to be force-fed by hand. *b*, Standard animal which started to develop its first bud.

Table 3 Head activator in cell fractions enriched for epithelial or nerve cells

Animal	Cell fraction	Cell composition (×10⁶) Total	Epithelial	Nerve	Content of head activator (BU)
Normal	Unseparated	90.0	20.0	4.5	400
	Epithelial enriched*	9.4	6.0	0.06	35
	Nerve-enriched†	5.3	1.9	2.8	320
Nerve-free	Unseparated	3.8	3.5	0	400

* 2–3 × 10⁶ cells were layered over an 8-ml linear Percoll gradient with a density range of 1.01–1.02, and separated by centrifugation at 300*g* for 10 min. Cells in the lower one-third of the gradient plus the pellet were pooled.

† Nerve cells were enriched using glycerol gradients as described earlier[5,12].

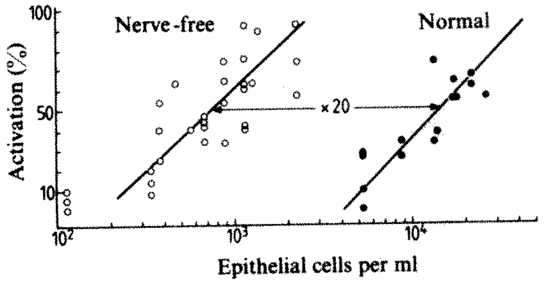

Fig. 2 Head activator in epithelial cells of nerve-free and normal hydra.

15.6 µm, respectively), a fraction could be obtained consisting mostly of epithelial cells and containing very few nerves (Table 3). From an epithelial-cell-enriched fraction a crude extract was prepared, and after purification on G-10, assayed for content of head activator. We found that it contained ~9% of the head activator present in unseparated cells (Table 3). Because only 1% of the nerve cells were represented in this fraction, we conclude that a minor portion of the head activator may be produced in normal animals also, by cell types other than the nerve cells, possibly epithelial cells. Figure 2 compares the content of head activator per epithelial cell in this isolated epithelial cell fraction from normal animals with that in nerve-free hydra. The results show that epithelial cells from nerve-free hydra contain at least 20 times more head activator than epithelial cells of normal animals. This is interpreted to mean that epithelial cells in normal animals may have the capacity to produce morphogenetic substances, but that this capacity is repressed in the presence of nerve cells.

Thus, our findings indicate that nerve-free hydra contain the same morphogenetic substances as normal hydra. One argument arainst a morphogenetic role for these substances was that they are neurohormones and are involved in a regulation imposed by the nervous system. The finding that no major difference exists in the morphogenetic properties between normal and nerve-free hydra supports the notion that hydra and morphogenetic substances in hydra are a good model for studying pattern formation in embryological systems.

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Expression of herpesvirus-induced antigens in human cervical cancer

Gordon R. Dreesman, Joyce Burek, Ervin Adam, Raymond H. Kaufman & Joseph L. Melnick

Departments of Virology and Epidemiology, and of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas 77030

Kenneth L. Powell & Dorothy J. M. Purifoy

Department of Microbiology, University of Leeds, Leeds, UK

Herpes simplex virus type 2 (HSV2) was first associated with human cervical cancer by seroepidemiologic studies over 10 years ago, but there has been no clearcut evidence confirming or refuting the role of the virus in this cancer. Expression of HSV antigens in cells transformed *in vitro* by the virus has been reported by several groups[1,2]. Evidence for the association of HSV antigens with human cervical cancer has recently been reviewed[3–5]; general antisera prepared against HSV virions or structural proteins were used by several groups to screen cells derived from the cancer and equivocal results were obtained. The tumour-specific antigens associated with three known oncogenic DNA viruses—adenovirus type 2, SV40, and Epstein–Barr herpesvirus—are DNA-binding proteins[5–8]. Therefore, proteins which bind DNA were isolated[9,10] from HEp-2 cells infected by HSV2, and monospecific antisera to two of these proteins (ICSP 11/12 and ICSP 34/35) were prepared. These rabbit antisera react specifically with the nuclei of HSV2-infected cells[11]. We show here that an HSV2-specific DNA-binding antigen was expressed in 38% of tissues with pathological findings of severe dysplasia or carcinoma. This therefore constitutes further evidence for HSV2 as a factor in human cervical carcinoma.

Prepared antisera to purified HSV2 virions (anti-HSV2) anti-ICSP 11/12 and anti-ICSP 34/35, and pre-inoculation rabbit serum were used to screen human cervical tissue for the presence of HSV-specific antigens. The cervical tissues were embedded in OCT compound (Lab-Tek) and quick frozen in a cryostat. Sections (10 µm) were cut for haematoxylin/eosin (HE) staining, and additional sections (6 µm) were cut for immunological staining procedures. All HE stained slides were double-blind coded for pathological examination. Adjacent sections were stained both by indirect immunoperoxidase (IP) staining and by indirect immunofluorescence (IF) staining[12]. All of the antisera (primary and goat anti-rabbit horseradish peroxidase or fluorescein conjugates) were adsorbed with uninfected HEp-2 cells and with rabbit liver powder before use.

Tissues obtained from 124 women were screened for the presence of HSV2-specific antigens using the above sera. the results are summarised in Table 1. Whereas anti-ICSP 34/35 gave a nuclear pattern of staining with infected HEp-2 cells, all of the positive reactions observed by screening biopsy material

Table 1 Staining of human cervical biopsy sections by indirect immunoperoxidase

Diagnosis	Total tested	No. negative	No. positive	Per cent positive
Normal	15	15	—	0
Chronic inflammation	6	6	—	0
Metaplasia	27	26	1	4
Dyplasia				
Mild	35	34	1	3
Moderate	20	18	2	10
Severe	11	7	4	36
Carcinoma *in situ* and invasive	10	6	4	40

The sections were stained with rabbit anti-HSV-specific ICSP 34/35 sera.

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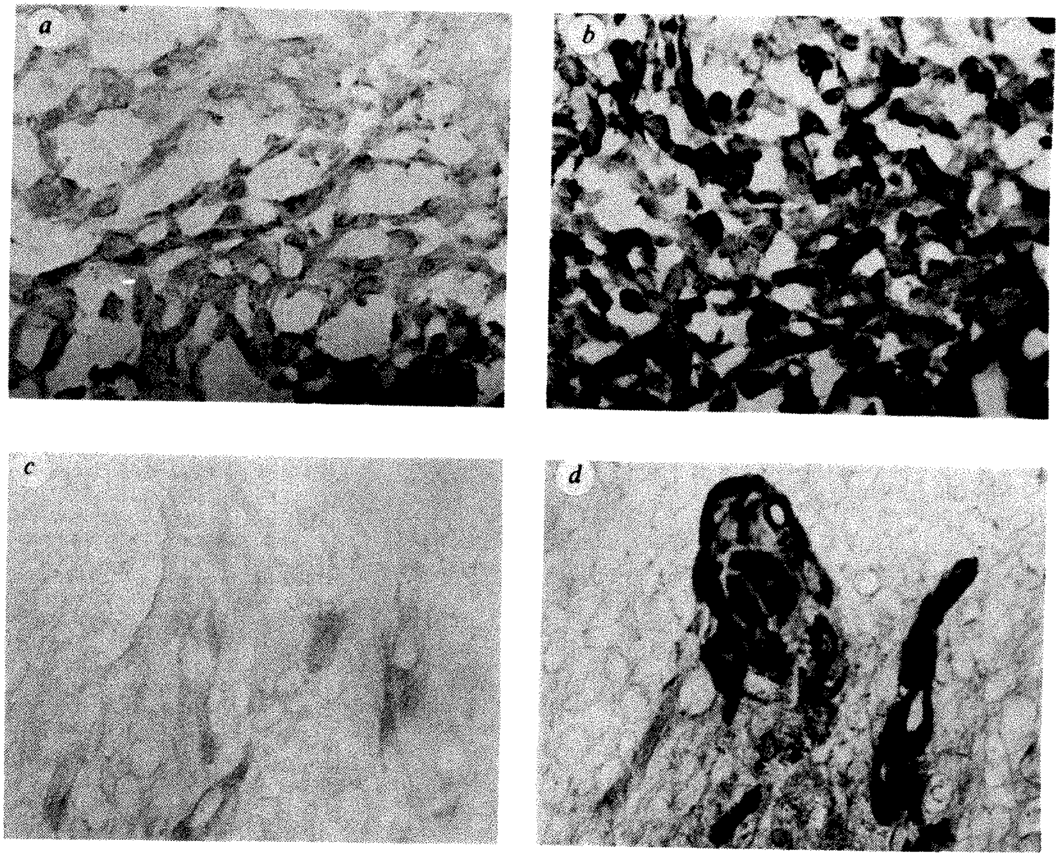
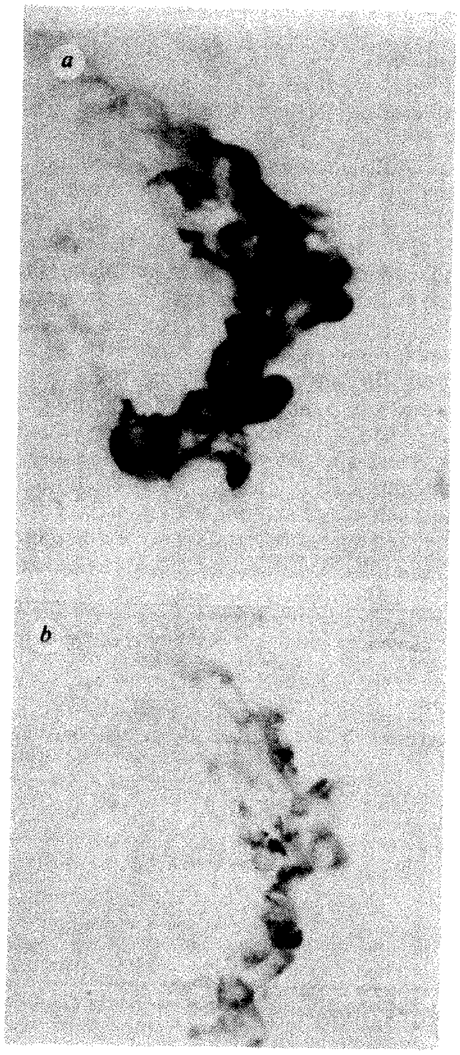


Fig. 1 Immunoperoxidase staining of human cervical tissues. *a* and *b*, Tissues were from a patient diagnosed as severe dysplasia; *c* and *d*, tissues were taken from a patient with carcinoma *in situ*. The primary antisera used were rabbit anti-ICSP 34/35 (*b* and *d*) and rabbit pre-inoculation (*a* and *c*). ×560.



were perinuclear and cytoplasmic. Typical patterns are shown in Fig. 1. This staining pattern is similar to that observed previously by Flannery *et al.*[2], who used an antiserum designated anti-VP143 to stain a number of HSV2-transformed cell lines. The VP143 HSV2 protein has the same molecular weight as ICSP 11/12. When the tissues were stained using the IF technique, the results were the same as those given in Table 1. If the tissues are divided into two groups (group I, those with diagnoses of normal, chronic inflammation, metaplasia, mild dysplasia, and moderate dysplasia; and group II, those with severe dysplasia, carcinoma *in situ* and invasive carcinoma), the following observations can be made: the incidence of positives in group I is 4 out of 103, or 4%, and in group II is 8 out of 21, or 38%. Similar but weaker patterns were observed when the majority of the above tissues were studied using anti-ICSP 11/12. However, no reactivity was seen using antiserum to HSV2 whole virions. Comparable results have been obtained by McDougall *et al.* (personal communication) using anti-ICSP 11/12.

The specificity of the reactions has been tested by adsorbing the anti-ICSP 34/35 sera with HSV2-infected HEp-2 cell lysates. The adsorbed sera were now negative when allowed to react with infected cells. The adsorbed sera were also tested with cervical tissue sections from seven positive patients. In six cases the reaction was now negative or significantly diminished (Fig. 2*a*, *b*). In the seventh case, the reaction was essentially unchanged. The six tissues in which the reaction was blocked or significantly diminished had a pathological reading of severe dysplasia or carcinoma. The seventh tissue, the reactivity of which was not altered by blocking, was a section from tissue diagnosed as metaplasia. The significance of these patterns is being investigated in greater detail.

One tissue with a pathology report of severe dysplasia gave nuclear staining by the IP staining procedure. However, this pattern was also noted when the tissue was reacted with the

Fig. 2 Immunoperoxidase staining of a human cervical tissue with a pathological finding of moderate dysplasia. The primary antiserum used was rabbit anti-ICSP 34/35 before (*a*) and after (*b*) adsorption with HSV2-infected cell lysates. ×560.

anti-HSV2 virion serum, as well as with an anti-HSV1 serum. There was only one localised area in the tissue that contained cells which gave this nuclear pattern of staining.

In summary, an HSV2-specific DNA-binding antigen was expressed in 38% of tissues with pathological findings of either severe dyplasia or carcinoma. This is of additional interest as these same tissues are negative when examined with an anti-virion HSV2 serum. The specificity of the reactivity for HSV DNA-binding protein antigen was demonstrated in that (1) pre-inoculation rabbit serum gave no reaction; (2) 21 tissues with no pathological changes were negative; (3) adsorption of the anti-ICSP 34/35 serum with HSV2-infected cell lysate blocked the reaction; and (4) adsorption with lysates of uninfected HEp-2 cells did not alter the reactivity. Our data lend support to a previous finding that about 60% of human cervical tumours contain HSV mRNA[13]. These fingerprints add further evidence for HSV2 as a factor in human cervical carcinoma.

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(ADP–ribose)$_n$ participates in DNA excision repair

Barbara W. Durkacz, Olusesan Omidiji, Douglas A. Gray & Sydney Shall

Biochemistry Laboratory, University of Sussex, Brighton,
Sussex BN1 9QG, UK

Chromatin proteins are covalently modified by at least five different processes; in no case has the precise physiological function been established. One of these post-synthetic, covalent modifications is effected by the enzyme poly(ADP–ribose) polymerase, which uses the coenzyme NAD$^+$ to ADP-ribosylate chromatin proteins[1–3]. The modification consists largely of mono(ADP–ribose), but long, homopolymer chains of (ADP–ribose) are also present. Various physiological functions have been suggested for (ADP–ribose)$_n$. Here we demonstrate that one function of (ADP–ribose)$_n$ is to participate in the cellular recovery from DNA damage. Specific inhibitors of poly(ADP–ribose) polymerase prevent rejoining of DNA strand breaks caused by dimethyl sulphate and cytotoxicity is enhanced thereby. The rejoining of strand breaks is prevented also by nutritionally depleting the cells of NAD.

Both radiation[4–6] and alkylating agents[7–10] lower the cellular NAD content. An explanation for this phenomenon was suggested when we showed that the nitrosourea, streptozotocin, both lowered cellular NAD content and raised the specific activity of poly(ADP–ribose) polymerase[9]. Treatment of cells with *N*-methyl-*N*-nitrosourea or with γ-radiation results in a profound drop in cellular NAD content, and this decrease is correlated with enhanced activity of poly(ADP–ribose) polymerase[10–12]. The enzyme activity also increases when permeabilised cells are incubated with either endogeneous[13] or exogeneous endonucleases[13,14]. Poly(ADP–ribose) polymerase activity increases in human lymphoblastoid cell lines after UV irradiation, and this response is abolished or delayed in xeroderma pigmentosum cells, which are unable to excise pyrimidine dimers[15]. Thus DNA fragmentation seems to activate poly(ADP–ribose) polymerase, thereby decreasing the cellular NAD content[13].

Four chemical classes of inhibitors of poly(ADP–ribose) polymerase are known; nicotinamides (such as 5-methylnicotinamide)[16–18], methylxanthines[10,11,19,20], thymidine[16,21] and aromatic amides such as benzamides (refs 21, 22, and G. Khan and S.S. unpublished results). The inhibition constant (K_i) for 3-aminobenzamide in isolated L1210 nuclei has been estimated to be 1.8 ± 0.2 μM (M. Purnell and W. J. D. Whish, personal communication). We have confirmed that 3-aminobenzamide is a very powerful, competitive inhibitor of poly(ADP–ribose) polymerase. The K_i in permeabilised[13] L1210 mouse leukaemic lymphoblast cells, at 26 °C is 4.4 ± 1.1 μM. This enzyme inhibitor is non-toxic to these L1210 cells at up to 3 mM, as shown by a cloning assay in soft agar. Two enzymes catabolise NAD in eukaryotes—NAD glycohydrolase (EC 3.2.2.6) and poly(ADP–ribose) polymerase. 3-Aminobenzamide does not inhibit NAD glycohydrolase in our system. As 3-aminobenzamide does not reduce the cloning efficiency of these cells, it is likely that it does not have profound metabolic effects of an unknown character in undamaged cells.

The cellular NAD content is lowered by dimethyl sulphate (DMS) in a dose-dependent manner (Fig. 1*a*), reaching the minimum level in 1 to 2 h of treatment. Poly(ADP–ribose) polymerase activity in nucleotide-permeable cells[13] after DMS treatment increases (threefold at 1 mM DMS) in a dose-dependent way and shows a peak of activity when the NAD content is dropping most rapidly (Fig. 1*c*).

At 1 mM, 3-aminobenzamide prevents the NAD decrease completely, while the non-inhibitor, 3-aminobenzoic acid, has no effect (Fig. 1*b*). The decrease in NAD induced by DMS is also inhibited by 5-methylnicotinamide, thymidine/deoxycytidine and theophylline, but not by nicotinic acid (data not shown). Neither 5-methylnicotinamide nor thymidine/deoxycytidine significantly inhibit NAD glycohydrolase[12]. The decrease in NAD content induced by DNA-damaging agents is mediated by poly(ADP–ribose) polymerase; thus (ADP–ribose)$_n$ may be implicated in some aspect of DNA repair.

We have therefore examined the kinetics of removal of single-strand breaks (this includes breaks produced by the alkaline conditions of the gradient) following treatment with DMS. Immediately after treatment, the DNA sediments as a broad peak in the middle of the gradient (Fig. 2*c*, *a*). At 40 min after removal of the DMS, the DNA is increased significantly in size (Fig. 2*c*), and by 80 min it is completely converted into a fast sedimenting complex near the bottom of the gradient, which is the position at which undamaged DNA sediments (Fig. 2*d*).

However, when the enzyme inhibitor 3-aminobenzamide (3 mM) is added to the cells following removal of DMS, conversion of the low molecular weight DNA to a fast-sedimenting material is almost completely inhibited (Fig. 2*a*, *b*). There is no observable increase in molecular weight of the DNA 40 min after removal of DMS (Fig. 2*a*). After 80 min, the DNA is increased slightly in size, but still sediments in the middle of the gradient (Fig. 2*b*). Even after 5 h the bulk of the DNA has not yet returned to its original size (Fig. 2*b*). Addition of 3 mM 3-aminobenzoic acid does not retard excision repair following DMS treatment (data not shown).

Other inhibitors of the polymerase also inhibit excision repair in these cells. Both 5-methylnicotinamide and thymidine (in the presence of deoxycytidine) are effective inhibitors of excision repair (data not shown). Thus we have demonstrated that inhibitors of the enzyme, poly(ADP–ribose) polymerase, inhibit excision repair in these cells.

If (ADP–ribose)$_n$ biosynthesis participates in the cellular recovery from DNA damage, then inhibition of (ADP–ribose)$_n$

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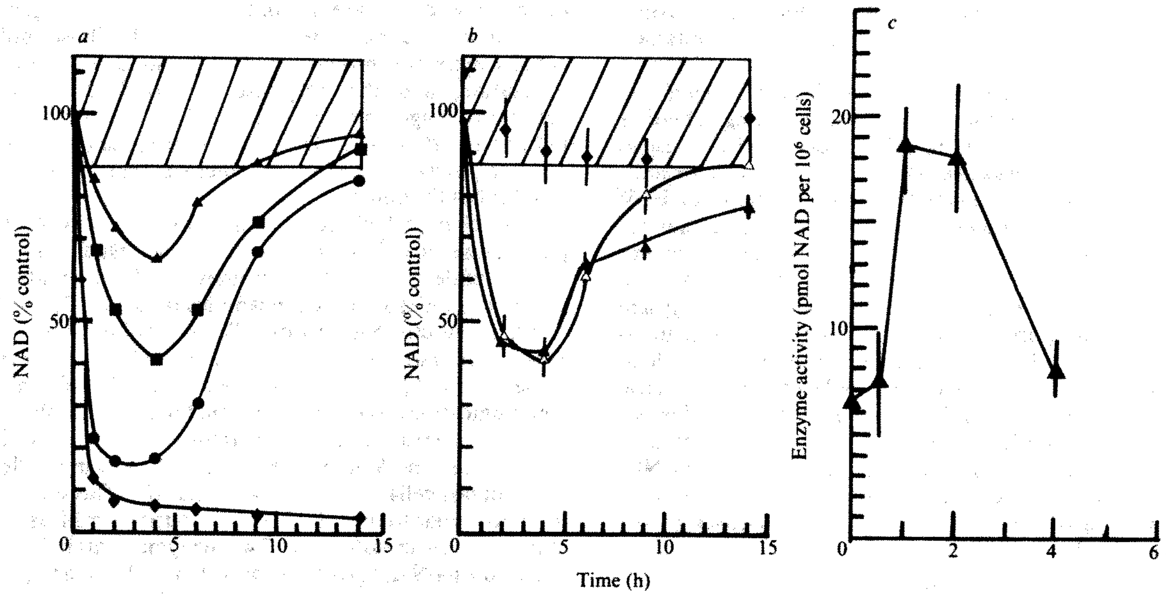

Fig. 1 The effect of DMS and of poly(ADP–ribose) polymerase inhibitors on the NAD content of L1210 cells. *a*, Cells were continuously treated with increasing doses of DMS, and samples were removed at intervals for NAD assay, washed once with 0.9% (w/v) NaCl, resuspended in cold 50% (v/v) ethanol, and then sonicated for 20 s (medium power, amplitude 1, MSE sonicator). Each sample was assayed for NAD in duplicate by a modification of the method of Nisselbaum and Green[12]. ▲, 10.5 μM DMS; ■, 52.6 μM; ●, 105.3 μM; ◆, 210.6 μM. *b*, L1210 cells were continuously treated with 53 μM DMS either alone (△) or in the continuous presence of either 2 mM 3-aminobenzamide (◆) or of 2 mM 3-aminobenzoic acid (▲). Samples were removed at intervals for NAD estimation[12]. *c*, Cells were treated continuously with 1 mM DMS and then the poly(ADP–ribose)polymerase activity was determined at the indicated times. Permeabilised cells[13] were cooled to 4 °C, collected by centrifugation in the cold and washed once with cold Pucks saline (containing 400 mg l^{-1} KH_2PO_4, 350 mg l^{-1} $NaHCO_3$, 8 mg l^{-1} NaCl, and 1 g l^{-1} glucose at *p*H 7.4). The cells were resuspended in a cold hypotonic buffer *p*H 7.8, (containing 9 mM HEPES, 4.5% (w/v) dextran, 1 mM EGTA, 4.5 mM $MgCl_2$, and 5 mM DTT). The cell concentration in this hypotonic buffer was $3–5 \times 10^7$ per ml. After 30 min incubation at 4 °C the cells were diluted 10-fold with an isotonic buffer *p*H 7.8 (containing 40 mM HEPES, 130 mM KCl, 4% (w/v) dextran, 225 mM sucrose, 2 mM EGTA, 2.3 mM $MgCl_2$ and 2.5 mM DTT). 250 μl of cells were incubated at 25 °C with 5 μl of ^3H-NAD (final concentration 2 μCi ml^{-1}, 100 μM). Triplicate samples were taken at 2-min intervals up to 8 min. The reaction was stopped with 4 ml of 20% (w/v) trichloroacetic acid (TCA). The samples were left at 4 °C for 4 h, filtered on to glass fibre disks and washed four times with 10% (w/v) TCA, and twice with acetone. The filters were dried and the radioactivity estimated in a toluene-PPO scintillant in a Beckman LS233 liquid scintillation counter. Enzyme activity was estimated by least squares regression analysis. The control level of poly(ADP–ribose) polymerase in these conditions was 6.63 ± 0.94 (s.d.) pmol min^{-1} per 10^6 cells (nine determinations).

biosynthesis would be expected to increase the toxicity (and perhaps also the mutagenicity) of DNA-damaging agents.

Exposure of L1210 cells to DMS for 1 h results in a dose-dependent cytotoxicity (Fig. 3). 90 μM DMS yields a 10% survival. However, the toxicity of DMS is greatly increased when 2 mM 3-aminobenzamide is present during both exposure of the cells to DMS and in the subsequent cloning assay. In these conditions 22 μM DMS is sufficient to give 10% survival; this represents a fourfold dose-enhancement. 3-Aminobenzoic acid (2 mM) has no effect on the cytotoxicity of DMS (Fig. 3). In addition, 5-methylnicotinamide, thymidine/deoxycytidine and theobromine potentiate the cytotoxicity of DMS (O.O. and S.S., manuscript in preparation).

This potentiation of cytotoxicity is observed also with several other monofunctional alkylating agents, and with representatives of each class of enzyme inhibitor (N. Nduka, O.O., A. A. Zia'ee and S.S., manuscripts in preparation).

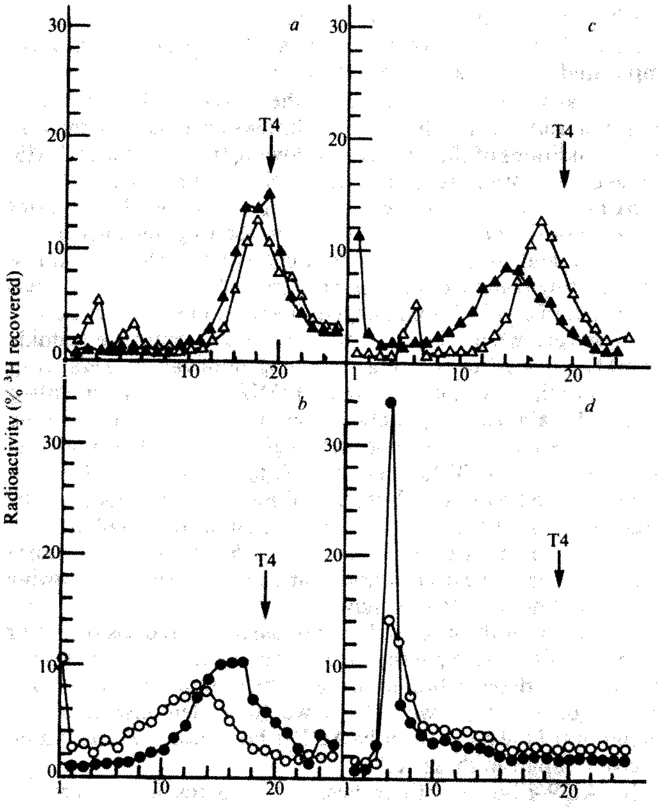

Fig. 2 The effect of 3-aminobenzamide on excision repair in L1210 cells. L1210 cells were prelabelled for 18 h (1.5 generations) with 1 μCi ml^{-1} ^3H-thymidine, 1 μM total thymidine. The radioactivity was washed out and the cells were treated with 100 μM DMS for 20 min. The cells were collected and resuspended in RPMI 1640 medium and incubated at 37 °C. Samples of 1 ml were removed at intervals, centrifuged and resuspended in cold phosphate-buffered saline and kept on ice. Samples of 25 μl ($1–2 \times 10^4$ cells) were taken for each time point and layered on a lysis layer (100 μl 2% (w/v) SDS, 10 mM EDTA) over a 4.8 ml to 20% (w/v) sucrose gradient (100 mM NaCl, 100 mM NaOH). The gradients were centrifuged for 1 h at 20 °C in a SW 50.1 Beckman rotor at 25,000 r.p.m. Fractions (12 drops) were collected on filter strips and the TCA-insoluble radioactivity was estimated. Time after removal of DMS: ■, 0 min; □, 40 min; ○, 80 min; ●, 300 min. The total radioactivity loaded onto the gradients was $\sim 2 \times 10^4$ c.p.m.; recovery was between 80 and 100%. *a*, The cells were exposed to DMS for 20 min, which was washed out, but 3 mM 3-aminobenzamide was added to the medium for the 300 min recovery period. *b*, The cells were exposed only to DMS. The arrow shows the position of a ^{14}C-labelled bacteriophage T4 DNA, single-stranded of molecular weight 55×10^6.

The evidence that (ADP-ribose)$_n$ biosynthesis participates in cellular recovery from DNA damage rests heavily on experiments with enzyme inhibitors. Although we show that all four classes of polymerase inhibitors are effective, the possibility remains that all these polymerase inhibitors might also coincidentally inhibit some aspect of DNA repair directly. Therefore, we have tested the ability of cells to carry out excision repair when the cellular NAD content is lowered by nutritional deprivation. NAD levels of cells may be depleted by growing them in a nicotinamide-free medium[23]. Even with quite low NAD content (10–20% of normal) the L1210 cells continue to grow and divide at the normal rate. The rate of incorporation of ^{3}H-thymidine by these NAD-depleted cells is the same as that of cells grown either in complete medium or in depleted medium supplemented with nicotinamide for 4 h. The NAD content of L1210 cells is 750 pmol per 10^6 cells; the NAD concentration is therefore about 1,000 μM. The K_m value for the DNAase-activated[24] polymerase in permeabilised cells[13] is 328 ± 127 μM. Thus, lowering of the NAD content should decrease the activity of the enzyme, and therefore inhibit DNA repair.

After exposure to 100 μM DMS, the DNA in NAD-depleted cells becomes fragmented (Fig. 4*a*), and not only is strand-rejoining inhibited, but the DNA is further decreased in size during the subsequent 80 min (Fig. 4*a*). This inhibition of recovery is alleviated if the cells are pre-incubated for 4 h in the presence of nicotinamide before DMS treatment (Fig. 4*b*).

NAD-depleted cells in the presence of hydroxyurea do not display unscheduled DNA synthesis after treatment with methylnitrosonitroguanidine[32].

The evidence that (ADP-ribose)$_n$ is involved in DNA repair can be summarised as follows. Both alkylating reagents and radiation lower cellular NAD contents, and they both elevate poly(ADP-ribose) polymerase activity. The drop in NAD content and the rejoining of strand breaks after treatment with DMS are inhibited by 3-aminobenzamide and the other poly(ADP-ribose) polymerase inhibitors, all of which potentiate the cytotoxicity of the alkylating agent. In addition, cells that are nutritionally deprived of NAD cannot efficiently execute excision repair. *N*-methyl-*N*-nitro-*N*-nitrosoguanidine lowers cellular NAD levels and increases intracellular poly(ADP-ribose) levels[33].

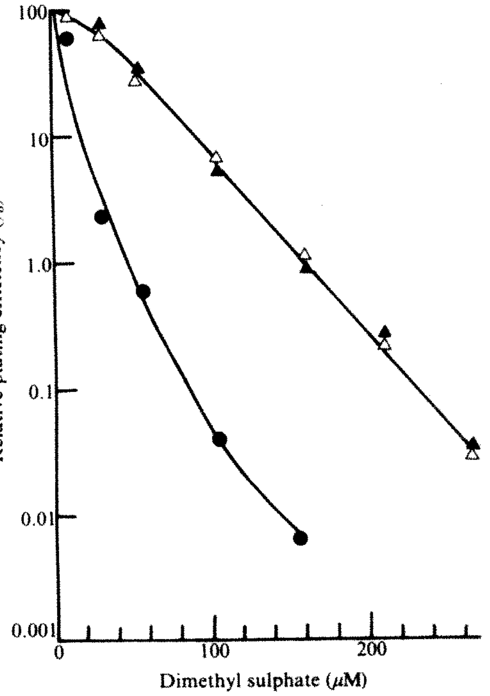

Fig. 3 The effect of 3-aminobenzamide on the cytotoxicity of DMS towards L1210 mouse leukaemia lymphoblast cells. Cells were treated with increasing doses of DMS alone for 1 h at 37 °C (△), or with DMS for 1 h in the continuous presence of either 2 mM 3-aminobenzamide (●) or 2 mM 3-aminobenzoic acid (▲). Cells were plated in 0.5% (w/v) agar in RPMI 1640 medium supplemented with 20% (v/v) horse serum in the absence or presence of the inhibitors, respectively. For DMS alone, the points are the means of nine experiments, each with five plates per point; for the other two situations the points are the means of two experiments each with five plates per point.

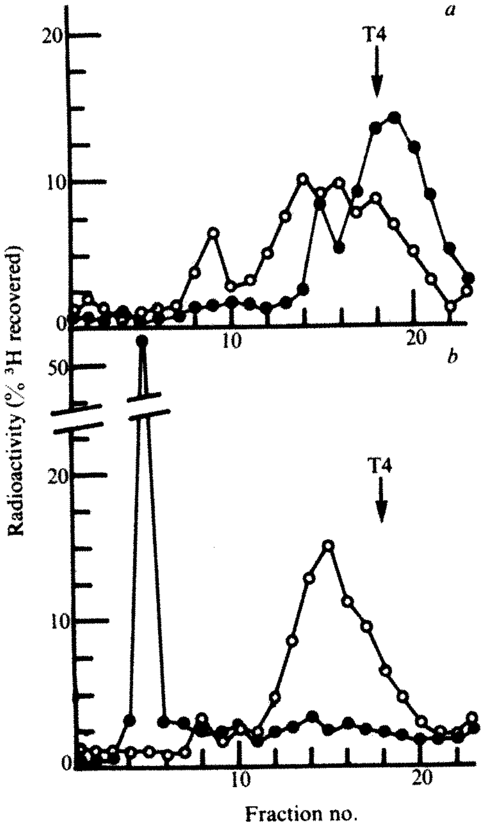

Fig. 4 The effect of NAD depletion on excision repair. Cells were grown for four generations in medium from which nicotinamide was omitted, and the serum was extensively dialysed against phosphate-buffered saline. NAD assays showed that the NAD content was 10 to 20% of the control levels. The culture was divided into two aliquots and nicotinamide was added to one to a concentration of 9 μg ml^{-1}. The cells were exposed 4 h later to 100 μM DMS for 20 min and then their ability to carry out excision repair was tested as described in the legend to Fig. 2. Time: ○, 0 min; ●, 80 min. *a*, NAD-depleted cells. *b*, Nicotinamide added back; the NAD level had returned to normal.

The mechanism involved when (ADP-ribose)$_n$ participates in the cellular recovery from DNA damage is not yet understood. Two general types of mechanism may be considered. A protein (enzyme) specifically involved in the molecular DNA repair mechanism may be activated or inhibited by ADP-ribosylation. For example, ADP-ribosylation of a Ca^{2+},Mg^{2+}-dependent endonuclease results in the reversible inhibition of nuclease activity[25]. Alternatively, ADP-ribosylation of either a specific or a nonspecific set of chromatin proteins would profoundly alter local chromatin structure. It has been suggested that (ADP-ribose)$_n$ may have cross-linking properties, or may be involved in extending and condensing chromatin[26–30]. Cell extracts from some complementation groups of xeroderma pigmentosum can excise pyrimidine dimers from naked DNA but not from chromatin[31]; this implies a need for chromatin structural alterations in excision repair.

Our results show that (ADP-ribose)$_n$ biosynthesis is required for efficient excision repair and survival, following damage with monofunctional alkylating agents. The dramatic synergistic potentiation of cell killing by alkylating agents and poly(ADP-ribose) polymerase inhibitors may be of use in the treatment of human leukaemia.

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Mutagenicity of carcinogenic methylating agents is associated with a specific DNA modification

R. F. Newbold, W. Warren, A. S. C. Medcalf & J. Amos

Chemical Carcinogenesis Division, Institute of Cancer Research,
Pollards Woods Research Station, Nightingales Lane, Chalfont St Giles,
Bucks HP8 4SP, UK

The carcinogenic potency of simple aliphatic alkylating agents such as the alkylnitrosamides and the alkylmethanesulphonates is positively correlated with their ability to alkylate the relatively weakly nucleophilic oxygen atoms in DNA, particularly the O^6-atom of guanine[1–4]. Differences in the spectrum of DNA alkylations produced by these agents can be rationalised on chemical grounds in that the electrophilic reactivity of the alkylating species determines the extent to which it will react at sites of weaker nucleophilicity[4–6]. Alkylation of the more strongly nucleophilic ring nitrogen atoms of the purine bases, which is the main site of reaction with all these agents, appears to be much less important in alkylation carcinogenesis. O^6-alkylation of guanine is likely to interfere with DNA base-pair hydrogen bonding[7] and is possibly the major DNA modification responsible for the induction of GC → AT transition mutations in bacteria and bacteriophage by alkylating agents[8–10]. Here, we have studied the effects of three methylating agents of contrasting carcinogenic potency on mammalian (V79 Chinese hamster) cells in culture. We report that the mutagenicity but not the cytotoxicity of each agent reflects its carcinogenicity and, furthermore, that the marked differences in mutagenicity are closely paralleled by differences in levels of O^6-guanine methylation.

The somatic mutation theory of cancer requires that all carcinogens be mutagens and that there be some quantitative relationship between their carcinogenicity and mutagenicity. To test this prediction, we have developed reliable and efficient methods for measuring carcinogen-induced gene mutation frequencies in cultured mammalian cells, based on classical drug-resistance markers, along with techniques for the isolation and chemical analysis of carcinogen-modified DNA. We have previously used the induction of 8-azaguanine-resistant (azg^r) and ouabain-resistant (oua^r) V79 Chinese hamster cells to define the relationship between carcinogenicity, mutagenicity and DNA reaction for various polycyclic hydrocarbon carcinogens[11–14]. In particular, *anti*-benzo(*a*)pyrene-7,8-dihydrodiol-9,10-oxide, the proposed ultimate carcinogenic metabolite of benzo(*a*)pyrene, was an exceptionally potent mutagen in this system[12]. Since V79 cells do not excise O^6-methylguanine from their DNA (ref. 15), they are also ideal for use in experiments designed to establish the role of this base in the mutagenicity and cytotoxicity of methylating agents. For the present investigation, we selected three methylating agents: dimethylsulphate (DMS) and methylmethanesulphonate (MMS) both relatively weak carcinogens and *N*-methyl-*N*-nitrosourea (MNUA), a highly potent carcinogen[16]. Quantitation of cytotoxicity and mutation induced by these compounds, coupled with high-pressure liquid chromatography of hydrolysed DNA from cells treated with radiolabelled MNUA and DMS, has enabled the modification specifically responsible for their mutagenic effects to be identified.

The results of several experiments in which the mutagenic effects of DMS, MMS and MNUA were compared in identical experimental conditions are summarised in Fig. 1. Parallel cultures of exponentially dividing V79 cells were exposed for 3 h to a range of concentrations of each compound, and replicate samples were plated for determination of induced cytotoxicity and frequency of azg^r and oua^r mutants; the experimental details are given in the legend to Fig. 1. With all three compounds a similar type of dose–survival curve was obtained, comprising a shouldered (quasi-threshold) region at low doses followed by a steeper portion where survival dropped rapidly with increasing dose. However, the dose range required to achieve this response varied approximately in the ratio 1:3:6 for DMS, MMS and MNUA respectively, the corresponding D_{37} values being 0.11 mM, 0.32 mM and 0.73 mM. The mutational response did not reflect the differences observed between the compounds in survival experiments; MNUA was by far the most potent agent at inducing both azg^r and oua^r clones. With all three compounds, dose–response curves for the induction of oua^r mutants were linear (above 10% survival where mutation frequencies could be measured most accurately) while those for azg^r mutants were cumulative. The contrasting mutagenicity of the agents was more clearly illustrated if induced mutation frequencies were plotted as a function of the logarithm of the corresponding percentage survival, as in Fig. 1. These plots were linear, as routinely found with other classes of mutagen[14,17]; lines of best fit were determined by least-squares linear regression analysis. The ratio of the slopes of these lines (mutagenic efficiency[14,18], or mutagenicity per lethal event) of MNUA relative to DMS, was approximately 15:1 for azg^r mutants and 9:1 for oua^r mutants. Although MMS was less potent than DMS as a mutagen in terms of applied dose (mutagenic effectiveness[14,18]) the two agents were indistinguishable when their mutagenicity was expressed as a function of induced cytotoxicity. This is explained by the fact that the rate of reaction of DMS is faster than that of MMS (half-time for reaction with DNA *in vitro* is 16 min compared with 5 h) although both agents share similar reaction mechanisms and produce similar proportions of the various methylated DNA products[4,19]. To confirm that only true mutants were scored in this series of experiments, mutant clones were isolated at random from plates and the cells grown in mass culture. All clones isolated remained fully resistant to the drug used to select them even when propagated for many generations in its absence. In addition, cells possessing the azg^r phenotype invariably had markedly reduced or undetectable amounts of the purine salvage enzyme hypoxanthine-guanine phospho-

Table 1 Extent of methylation of V79 cell DNA at major sites following exposure of cells in culture for 3 h to ^{14}C-DMS and ^{14}C-MNUA

Compound	Biological effects		Extent of methylation of DNA (μmol per mol DNA P)		
	Survival (%)	azg^r mutation frequency per 10^5 survivors)	7-methylguanine	3-methyladenine	O^6-methylguanine
DMS (8 μg ml^{-1})	82	10	48.1	8.8	ND
DMS (15 μg ml^{-1})	58	34	92.4	12.0	0.5
MNUA (30 μg ml^{-1})	81	148	50.3	5.4	7.5
MNUA (60 μg ml^{-1})	48	373	102.0	6.2	14.4

In these experiments, each 175 cm^2 plastic tissue culture flask (Nunc UK) was inoculated with 10^7 exponentially growing V79/4-K$_1$ cells in 50 ml DMEM. After incubation at 37 °C for 24 h, groups of five such cultures (about 2×10^8 cells) were treated either with ^{14}C-MNUA or ^{14}C-DMS (Radiochemical Centre, Amersham) of specific radioactivity 11 mCi mmol^{-1} and 18 mCi mmol^{-1} respectively. These doses were chosen to give approximately 80% or 50% survival (see Fig. 1). Immediately before treatment, stocks of the compounds were prepared at 250 times the required treatment concentration in DMSO which had been dried over Molecular Sieve (Fisons). Control cultures were treated with 0.2 ml DMSO alone. After 3 h exposure to the compounds the cells were detached from the flasks with trypsin/EDTA and samples taken for assay of survival and mutation frequency as described in the legend to Fig. 1. The remaining cells were washed twice in phosphate-buffered saline by centrifugation and resuspension. DNA was isolated from cell pellets by a modification of the method of Kirby[24]. Prior to chromatography, DNA samples were dissolved in 0.02 M phosphate buffer and hydrolysed in 0.1 M HCl at 70 °C for 30 min to liberate normal and methylated purine bases. The products of hydrolysis were chromatographed, together with marker alkylated bases, using a Waters ALC GPC 204 liquid chromatograph equipped with a Model U6K injector, a Model 440 ultraviolet detector (Waters Associates) and two columns (25 cm × 0.9 cm) of Partisil-10 SCX cation exchanger (Whatman) connected in series with a precolumn of the same material. Separation of methylated purine bases was achieved by elution for 40 min (4 ml min.$^{-1}$) with a gradient running from 0.02 M ammonium formate to 0.2 M ammonium formate, *p*H 4.0, in 8% (vol/vol) methanol[21]. Values for the extent of methylation at various DNA sites (expressed as μmol per mol DNA P) were calculated from the radioactivity associated with the respective peaks and the specific radioactivity of the methylating species. The amount of DNA analysed was calculated from the ultraviolet absorbance of column fractions containing adenine and guanine. The resolution of O^6-MeGua was improved if the fractions containing this base were pooled, rotary evaporated at *p*H 8.0 and rechromatographed on an ODS μ-Bondapak column eluted with a gradient running from 5% methanol: 95% 0.05 M ammonium formate (*p*H 4.6) to 55% methanol: 45% 0.05 M ammonium formate. ND = not detectable.

ribosyl transferase (HPRT; EC 2.4.2.8)[20]. These results will be reported fully elsewhere.

In order to investigate observed differences in mutagenic efficiency at the DNA level, the scale of experiments was increased to permit sufficient quantities of carcinogen-modified V79 cell DNA to be isolated and analysed. Cells were exposed to ^{14}C-MNUA and ^{14}C-DMS labelled in the methyl groups. Doses of each compound were chosen with the aim of reducing cell survival to either 80% or 50% of the control plating efficiency. After sampling cells for analysis of survival and mutation frequency, DNA was isolated, hydrolysed and methylated products separated by high-pressure liquid chromatography as described previously[21]. Table 1 summarises the results of typical experiments. At approximately equal levels of cytotoxicity the overall levels of DNA methylation were similar for DMS and MNUA and were proportional to the dose applied. Mutation frequencies (azg^r) at a given dose were typical of those found in the initial studies with unlabelled compounds. As expected[4], chromatography revealed three major methylated DNA products: 7-methylguanine (7-MeGua) 3-methyladenine (3-MeAde) and, in the case of MNUA, O^6-methylguanine (O^6-MeGua). With DMS, the latter product was detectable only with the more toxic treatment (15 μg ml^{-1}; 58% survival) and then was only present at one-thirtieth the level found with an equitoxic dose of MNUA. This was the only difference observed between the compounds which could explain their contrasting mutagenic potency. The ratios of the various products did not vary significantly from those previously reported for DNA which had been exposed to the same agents *in vitro* or *in vivo*[2,4,19].

A comparison of MNUA- and DMS-induced minor alkylation products, which were not readily detectable at the relatively low extents of methylation where mutagenesis could be measured accurately, was investigated by exposing calf thymus DNA (Sigma) *in vitro* to ^{14}C-labelled compounds. Quantities of highly methylated DNA were obtained from these experiments which permitted more sensitive analysis of alkylation products to be carried out. The results of the analysis are shown in Table 2. The relative proportions of the three major products (7-MeGua, 3-MeAde and O^6-MeGua) were similar to those determined in V79 cells (Table 1) which confirmed that the reaction of these agents with cellular DNA can be represented, both qualitatively and quantitatively, by such a model system. Moreover, with MNUA, there was again no evidence of any product other than O^6-MeGua which could account for its potent mutagenic activity.

We were not surprised by the lack of a perfect quantitative relationship between the extent of O^6-guanine methylation and the number of mutants induced by DMS and MNUA. Such a correlation would only be expected if, as seems unlikely, all methylation products other than the O^6-MeGua (that is the majority of DNA modifications) totally lacked mutagenic effect. Recent studies in this laboratory (J. R. Connell and A.S.C.M., unpublished results) indicate that most chromosomal aberrations induced in V79 cells by these agents are not due to O^6-MeGua, since similar numbers of aberrations are encountered at equivalent levels of total DNA methylation and cytotoxicity. Although gross damage of this type would not be expected to be particularly efficient at inducing viable azg^r and oua^r mutants in comparison with a miscoding base, it could make

Table 2 Extent of methylation of calf thymus DNA at various sites following treatment *in vitro* with ^{14}C-DMS and ^{14}C-MNUA

Compound	Methylated DNA products (% of total)							
	3-MeGua	7-MeGua	O^6-MeGua	1-MeAde	3-MeAde	7-MeAde	O^2-MeCyt	Apurinic acid
DMS (expt 1)	1.3	70.9	0.3	1.0	14.6	2.0	ND	9.8
DMS (expt 2)	1.2	74.5	0.4	1.1	15.2	1.5	ND	6.1
MNUA (expt 1)	1.0	70.0	7.7	0.7	8.0	0.8	0.1	12.0
MNUA (expt 2)	1.0	64.8	7.2	0.7	9.5	1.4	0.1	15.2

Solutions of calf thymus DNA (5 mg DNA ml^{-1}) in 0.02 M sodium cacodylate buffer (*p*H 6.9) were treated with ^{14}C-DMS (6 μCi ml^{-1}; 60 mCi mmol^{-1}) or ^{14}C-MNUA (1.8 μCi ml^{-1}; 11 mCi mmol^{-1}) for 1 h at 37 °C with frequent shaking. Immediately following treatment, the DNA was precipitated by the addition of 1/8 vol. 2.5 M sodium acetate and 1½ vols 2-ethoxyethanol. Hydrolysis and chromatography of the DNA were performed as described for Table 1. The values shown are the means of three separate determinations and the extent of methylation of various DNA sites is expressed as a percentage of total DNA methylation.

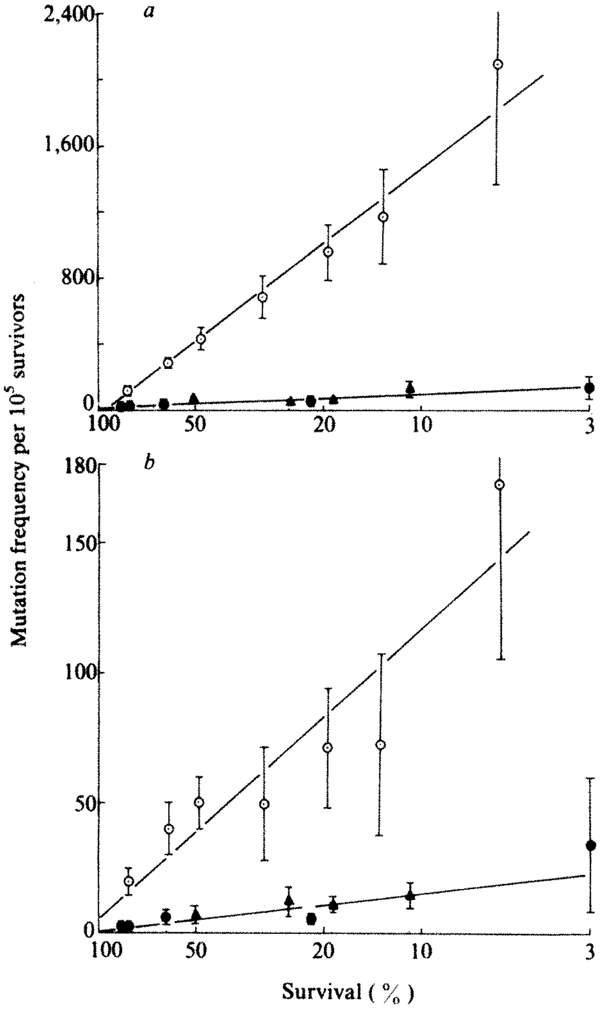

Fig. 1 Mutagenicity of DMS (●), MNUA (○) and MMS (▲) in V79/4-K_1 cells plotted as a function of percentage survival; *a*, azg^r, *b*, oua^r. The data, derived from several individual dose–response experiments, were expressed in this way to facilitate direct comparison between the agents in terms of their mutagenic potency per lethal event (mutagenic efficiency[14,18]). The advantages of this cell line for studies of mammalian cell mutagenesis have been discussed previously[11–14]. A clone of V79/4 designated V79/4-K_1, selected for its high plating efficiency and its ability to form diffuse microcolonies, was used throughout the course of this work. The latter characteristic allows the efficient recovery of mutants *in situ* which obviates the need to replate cells following each mutation expression period. In some experiments, both methods of selection were used and, invariably the highest mutant frequencies were observed with the *in situ* technique. In all experiments, 75 cm^2 plastic cell culture flasks (Nunc UK) containing 30 ml of Dulbecco's modified minimal essential medium (DMEM) with 10% fetal calf serum (Gibco) were each seeded with 2×10^6 cells grown from a clonal stock frozen in liquid nitrogen. After incubation at 37 °C for 24 h the resulting exponentially growing cultures were treated with various doses of the compounds added in 0.1 ml dry dimethylsulphoxide (DMSO). Control cultures were treated with 0.1 ml DMSO alone. After treatment at 37 °C for 3 h the cells were detached from each flask with trypsin-EDTA and the density of the resulting single-cell suspension estimated with a haemocytometer. For the determination of induced toxicity, 2×10^2 cells, and for mutation frequency, 5×10^4 cells, were plated in 5 cm and 9 cm plastic tissue culture dishes (Sterilin) respectively and incubated at 37 °C in a humidified atmosphere of 10% CO_2 : 90% air. Selection of azg^r (HPRT deficient) or oua^r (ATPase modified) mutants was effected by adding 8-azaguanine or ouabain to the plates such that the final concentration of the drug, in the medium, was 0.2 mM or 1 mM respectively. To achieve maximum recovery of mutants, a complete expression time curve was constructed for each dose of methylating agent. In practice, 5–10 replicates were treated with the selective agent at each of five expression time points ranging from 0–120 h after plating. Survival plates were stained after 7 d of incubation, mutation plates 3 d later, and colonies of more than about 50 cells, with observable mitotic figures, were scored. The plating efficiency of the cells was taken into account when calculating mutation frequencies. The values shown in the figure are derived from at least two separate experiments and are those obtained at peak expression times which ranged from about 48 h for minimally lethal doses to 96 h for the more toxic treatments. However, in all cases, maximum frequencies were observed after the cells had undergone an average of 4–5 divisions. Control frequencies did not exceed 4×10^{-5} per survivor. Standard deviations, indicated by bars attached to symbols, were calculated from the variance of the ratio of the mean numbers of colonies on mutation and survival plates; where bars are absent the standard deviation lies within the area of the symbol. Lines of best fit were obtained from DMS and MNUA data by least-squares linear regression analysis performed on individual mutation plate scores.

a major contribution to the overall mutagenicity of DMS or MMS which produce little O^6-MeGua. Indeed, there is evidence that, in bacteria, MMS is mutagenic by two distinct mechanisms, one of these dependent on the integrity of the *lexA*$^+$ (*exrA*$^+$)-mediated error-prone repair pathway[22]. Some insight into the mechanisms of alkylating agent mutagenesis in mammalian cells may be gained by detailed examination of the dose–response curves for mutation induction. The cumulative curves obtained in the present study for azg^r mutagenesis could imply the existence of two mutagenic mechanisms, one (presumably O^6-MeGua miscoding) predominating at low doses[23]. These curves can be described adequately by equations of the general form $M = aD + bD^2$ (M = azg^r mutation frequency per 10^5 survivors and D = dose in $\mu g\,ml^{-1}$) and for DMS and MNUA were calculated as $M = 1.30D + 0.13D^2$ and $M = 5.71D + 0.03D^2$ respectively. Interestingly, if we restrict our comparison of mutagenic activity to the linear terms of these equations (that is as D approaches 0) then the numbers of mutants calculated per μmol O^6-MeGua per mol DNA phosphorus (31 in the case of DMS and 24 for MNUA, extents of methylation estimated from data in Table 1) were very similar for the two agents.

Much remains to be understood about the relationship between specific DNA modifications and the various biological effects of the simple alkylating carcinogens. Nevertheless, the results described here strongly suggest that O^6-MeGua is responsible for the vast majority of gene mutations produced in mammalian cells by carcinogenic methylating agents. Moreover, the correlation between their mutagenicity in mammalian cells and their documented carcinogenic potency provides further support for the somatic mutation theory of cancer.

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Post-transcriptional control of coordinated ribosomal protein synthesis in *Escherichia coli*

Mats O. Olsson & Kirsten Gausing

Department of Molecular Biology and Plant Physiology, University of Aarhus, DK-8000 Århus C, Denmark

The synthesis of the approximately 50 different ribosomal proteins (r proteins) is very well coordinated[1] in *Escherichia coli*. As the r-protein genes are arranged in many different operons, placed at separate locations on the *E. coli* chromosome[2–5], it is not obvious how this coordination is maintained. The first indication that special controls are involved in the coordination came from the observations that merodiploid strains containing F′ factors with some of the ribosomal genes also synthesise the r proteins in balanced amounts[6,7]. The overall regulation of r-protein synthesis in response to changes in growth conditions is primarily mediated by changes in the rate of transcription of the r-protein genes[8]. To investigate whether the gene dosage control seen in merodiploid strains is also transcriptional in nature or whether other mechanisms are involved, we compared the transcription of r-protein mRNA in haploid and merodiploid strains. It was found that the rate of transcription of the r-protein mRNA from the *str–spc* cluster of genes changes in proportion to the gene dosage and it is concluded that the expression of r-protein genes is adjusted by post-transcriptional control.

About one half of the r-protein genes map in the *str–spc* region at 72 min on the *E. coli* chromosome and the genes are arranged in four separate transcriptional units[2]. All the r-protein genes in the *str–spc* region are present on the F′ factor KLF41 and most of the experiments were carried out with a pair of strains, with and without KLF41. Relative rates of synthesis and amounts of r-protein mRNA were measured using different types of hybridisation assays. As probes for different classes of mRNA DNA from the four transducing λ phages was used. λ*dfus*2 and λ*dspc*1 carry 27 (all) and 14 r-protein genes from the *str–spc* region, respectively[2]. As control for r-protein mRNA synthesis from genes not duplicated in the merodiploid strain, DNA from a transducing phage, λE3rp9, carrying r-protein genes from the 88-min region of the *E. coli* chromosome was used. The phage λE3rp9 was constructed *in vitro* and has the genes for L10, L11 and parts of the genes for L1 and L12 (K. G., in preparation). Nonspecific hybridisation was assayed with λ*dtrk* DNA. The phage λ*dtrk* is very similar to λ*dfus*2 and λ*dspc*1 but it does not carry any ribosomal genes[2].

For measurements of relative transcriptional activities of the r-protein genes, cultures were labelled in RNA for a period which was short relative to the half life of the unstable RNA fractions. The RNA was hybridised to excess DNA in conditions where the efficiency of hybridisation is better than 90% (Fig. 1). The relative rate of r-protein mRNA transcription from the *str–spc* region is nearly doubled in the F′-containing strain compared with the haploid strain whereas the L1–L12 protein mRNA is transcribed at similar rates in the two strains (Table 1). The gene dosage effect is the same for the complete and half of the *str–spc* region as *spc*-specific mRNA transcription is increased by the same factor as the *fus*-specific mRNA. The precise increase in the gene dosage in the KLF41-containing strain is not known. F′ factors are found in one or two copies per chromosome depending on the F′ factor and possibly the growth conditions[9]. The relative gene dosage (number of copies of gene *N* per genome equivalent) for the *str–spc* region on the chromosome is 1.15 calculated from the growth rate and the map position[10].

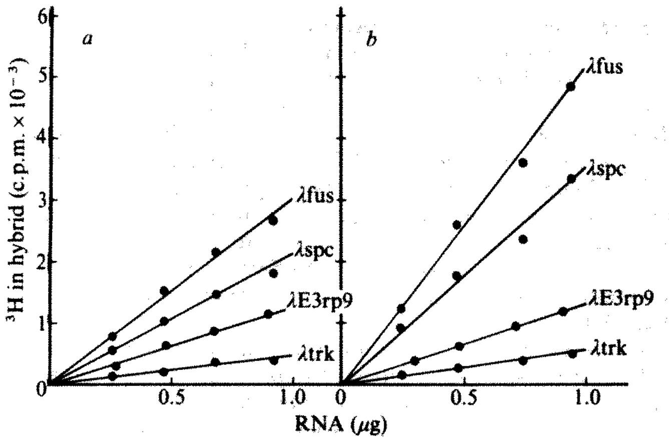

Fig. 1 Hybridisation analysis of pulse-labelled RNA from JC1553 and KLF41/JC1553. JC1553 (ref. 13) (*a*) and KLF41/JC1553 (ref. 14) (*b*) were obtained from N. Fiil. The two strains were grown exponentially at 37 °C in AB glucose minimal medium supplemented with a balanced mixture of 20 amino acids, the growth rates were 1.03 generations per h for JC1553 and 0.95 generations per h for KLF41/JC1553. At a density of 2×10^8 cells ml^{-1} the cultures were labelled for 45 s with [5, 6–^{3}H]uridine (40 μC ml^{-1} culture, specific activity 40 Ci mmol^{-1}) and the cells were collected on ice with Na-azide (10 mM). The RNA was extracted and hybridised to nitrocellulose filters with 10 μg of DNA from the transducing phages indicated as described previously[8]. Various amounts of RNA (0.25–1.0 μg) were hybridised and the values shown are averages of duplicate samples. The specific activity was 91,000 c.p.m. μg^{-1} for the JC1553 RNA and 88,000 c.p.m. μg^{-1} for the KLF41/JC1553 RNA.

The effect of changing the gene dosage for half of the r proteins in the *str–spc* region was measured by comparing the relative rates of transcription of r-protein mRNA in strains lysogenic for λ*dspc*1 and λ*dtrk*1. λ*dspc*1 contains two r-protein transcriptional units complete with the promoter regions (14 r-protein genes total)[2]. In the λ*dspc*1 lysogen the extra set of

Table 1 Relative rate of r-protein mRNA transcription

	% Of total radioactivity			Diploid/haploid		
Strain	L1–L12 mRNA	*spc* mRNA	*fus* mRNA	L1–L12 mRNA	*spc* mRNA	*fus* mRNA
JC1553	0.79	1.66	2.58			
KLF41/JC1553	0.73	2.92	4.57	0.9	1.8	1.8
NO1294 (λ*dtrk*)	0.51	1.36	2.36			
NO1267 (λ*dspc*1)	0.48	2.10	3.14	0.9	1.5	1.3

The per cent of the radioactivity in pulse-labelled RNA from JC1553 and KLF41/JC1553 that hybridises to four different types of DNA was calculated from the slopes of the curves in Fig. 1. Subtraction of the per cent radioactivity in λ*dtrk* DNA hybrid yields per cent radioactivity specific for the r-protein genes on λE3rp9 (genes for part of L1, L11, L10, and part of L12), λ*dspc*1 (14 r-protein genes) and on λ*dfus*2 (27 r-protein genes). NO1294 and NO1267 (from R. Jaskunas) are strains lysogenic for λ*dtrk* and λ*dspc*1. These strains were grown at 30 °C in AB glucose minimal medium; the growth rates were 0.78 generations per h for NO1294 and 0.74 generations per h for NO1267. RNA was labelled and analysed as described in Fig. 1 and results were evaluated as the results for JC1553 and KLF41/JC1553.

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r-protein genes is probably located at λ*att* at 11 min on the *E. coli* chromosome, in which case the gene dosage of the *spc* genes in the λ*dspc*1 lysogen is 1.7 relative to that in the λ*dtrk* lysogen. The increase in the relative rate of transcription from the duplicated genes is 1.5 (Table 1), which is seen directly from the *spc* mRNA results. The *fus* mRNA results are compatible with a 1.5-fold increase in the rate of transcription from the genes in the *str–spc* region that are duplicated in the λ*dspc*1 lysogen and identical rates of transcription from the genes that are not duplicated. A similar gene dosage effect on the rate of transcription of r-protein mRNA has been reported for a related λ*dspc*1 lysogen[11].

The JC1553 pair of strains differ from the pair of lysogens not only with respect to the mode of duplication of the *r*-protein genes. JC1553 is *recA* which is reflected in the relative low growth rate (see Fig. 1 legend), whereas the lysogens are *recA*$^+$. However, analysis of both sets of strains leads to the conclusion that the rate of transcription of the genes in the *str–spc* region is nearly proportional to the gene dosage. As the rate of synthesis of the r proteins in the merodiploid cells is well coordinated and identical to the rate of synthesis in the haploid cells[6,7,11], it follows that the yield of peptides per round of transcription is reduced specifically for the mRNA from the duplicated genes.

We then asked if this decrease in translation efficiency is reflected in the stability of these mRNAs. When the relative rate of transcription of a class of mRNA is known the average physical half life can be deduced from estimates of the amounts of mRNA. The relative amounts of r-protein mRNA were therefore determined in JC1553 and KLF41/JC1553. In one experiment the two strains were labelled uniformly in RNA and the per cent radioactivity in different classes of r-protein mRNA was measured by hybridisation to excess DNA. Table 2 shows that the merodiploid strain contains elevated levels of mRNA from the duplicated regions but the genes dosage effect is not as pronounced as for the rates of transcription of these mRNA species (Table 1). In another experiment pulse-labelled RNA was hybridised to limiting amounts of DNA. When DNA–RNA hybridisation approaches saturation a linear curve is obtained in a reciprocal plot (Fig. 2) and it has been shown that the amount of DNA-specific RNA is inversely proportional to the slope of the line[12]. Also this experiment shows that a duplication of r-protein genes is accompanied by a significant increase in the amount of the corresponding r-protein mRNA. A smaller increase (20%) in the amount of *spc* mRNA was seen in a strain lysogenic for a λ*dspc* phage[11], which probably reflects that the increase in r-protein gene dosage is smaller in the lysogen than in the KLF41-containing strain.

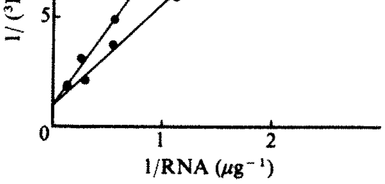

Fig. 2 Relative amounts of r-protein mRNA determined by hybridisation to limiting amounts of DNA. Different amounts of pulse-labelled RNA (0.4–7.0 μg) from JC1553 and KLF41/JC1553 (the same samples as used in Fig. 1) were hybridised to nitrocellulose filters with 0.5 μg λ*dtrk* DNA and 0.5 μg λ*dfus* DNA. Values on the ordinate represent c.p.m. hybridised to λ*dfus*2 DNA corrected for c.p.m. hybridised to λ*dtrk* DNA. The reciprocal of the slope for KLF41/JC1553 is 1.6 × the reciprocal of the slope for JC1553.

Table 2 Amounts of r-protein mRNA

Strain	JC1553	KLF41/JC1553
Total c.p.m.	154,000	158,000
c.p.m. In hybrid:		
λ*dtrk* DNA	510	575
λE3rp9 DNA	637	685
λ*dspc*1 DNA	858	1,098
λ*dfus*2 DNA	1,259	1,591
% In mRNA:		
L1–L12	0.08	0.07
spc	0.23	0.33
fus	0.49	0.64
Diploid/haploid:		
L1–L12 mRNA	0.9	
spc mRNA	1.44	
fus mRNA	1.31	
fus mRNA	1.6 (from Fig. 2)	

JC1553 and KLF41/JC1553 were grown exponentially at 37 °C in MOPS minimal medium with low phosphate (0.2 mM). The growth rate was 0.76 generations per h for JC1553 and 0.73 for KLF41/JC1553. At a density of about 6×10^6 cells ml^{-1} ^{32}P was added to the cultures (10 μC ml^{-1}) and 5 generations later (at 2×10^8 cells ml^{-1}) the cells were collected. RNA was extracted and duplicate portions of 0.5 μg and 1.0 μg were hybridised to nitrocellulose filters with 10 μg DNA. The c.p.m. in hybrid listed represent average values.

As the rate of transcription of the *str–spc* mRNA is increased more than the amount of the *str–spc* mRNA in the merodiploid strain, it follows that the average physical half life of the mRNA from the duplicated r-protein genes is somewhat shorter than in the haploid strain. However, the merodiploid strain does contain an excess of mRNA sequences from the duplicated r-protein genes.

In conclusion we have found that the rate of transcription from the r-protein genes in the *str–spc* region increases in proportion to the gene dosage when extra copies of these genes are supplied by an F′ factor or a transducing λ phage. As the rate of synthesis of the r proteins is unaffected by such changes in gene dosage[6,7] it shows that post-transcriptional controls adjust the rate of r-protein synthesis.

On the basis of similar observations it was recently proposed that the post-transcriptional control acts by inactivation and degradation of the excess of r-protein mRNA[11]. The results available so far are, however, equally compatible with mechanisms that involve blockage of translation of intrinsically active r-protein mRNAs. The increased lability of overproduced mRNAs could then be the consequence rather than the cause of their lower degree of participation in protein synthesis. The precise coordination of r-protein synthesis in normal cells, coupled with the recent observations that the r-protein gene clusters are spread over most of the chromosome[2–5], suggests that post-transcriptional control may be evolved in normal cells in order to compensate for the successive replication of the different r-protein genes during the cell cycle.

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MATTERS ARISING

Hybridisation of scDNA does not lead to overestimates of mRNA complexity

THE contention of Kiper[1], that complexity estimates of mRNA populations measured by hybridisation with single copy DNA (scDNA) are routinely overestimates, is not supported by previously published data and additional data we provide here. Kiper argues that selective loss of single copy sequences (cryptic unique sequences) occurs when scDNA is isolated from randomly sheared DNA because they are adjacent to repetitive sequences. He contends that the loss is selective for sequences which do not code for messenger RNA, and hence scDNA is enriched in sequences which code for mRNA. Thus complexity measurements on mRNA based on scDNA hybridisation result in overestimates.

From data on the sea urchin genome[2] it can be calculated that ~19% of the total genomic DNA might be present as 'cryptic' scDNA when 450-nucleotide fragments are used in the preparation of scDNA. Assuming all of the cryptic scDNA to be non-code sequence, the resulting scDNA would be enriched for code sequences such that saturation hybridisation data might yield a complexity estimate that is too high by a factor of ~1.3, which is less than suggested by Kiper. A more important consideration is the likelihood that code sequences are extensively represented in cryptic scDNA. For example 80–100% of the mass of the mRNA in sea urchin embryos is complementary to scDNA sequences which are adjacent to interspersed repetitive sequences[3]. If this is also true for complexity, and if the sequence organisation of sea urchin DNA is typical of eukaryotic genomes, then cryptic scDNA would contain code sequences and may even be somewhat enriched for these sequences. Whatever the case, a portion of the cryptic scDNA is expected to be code sequences. We have also found, using S_1 nuclease, that only about 10% of mouse DNA considered to be repetitive by hydroxyapatite (HAP) analysis of renaturation kinetics may be cryptic scDNA. Thus the extent of code sequence enrichment of scDNA suggested by Kiper seems unlikely.

Kiper's second point, that assay of DNA/RNA hybrids by HAP chromatography leads to overestimates due to the non-base-paired intervening sequence being present in DNA/RNA hybrids, is an important consideration in light of recent studies which suggest that several known structural genes are discontinuous in the genome[4–6], and perhaps, as well, a large number of unidentified mRNA coding sequences[7]. However, values obtained by HAP and S_1 nuclease assays of hybridised scDNA are similar (Table 1). It is evident from this comparison that a negligible portion of the hybridised scDNA is not base paired, as has been previously reported[8]. Despite these results we concur that S_1 nuclease method of assay is preferable to the use of HAP for reasons presented elsewhere[9].

From the aspects mentioned above Kiper suggests that the discrepancy between the scDNA and the cDNA methods of estimating the complexity of mRNA populations is largely due to gross overestimates generated by the scDNA method. From our results we find the discrepancy to be due more to the inaccuracy of the $C_0t\frac{1}{2}$ determinations for the most complex class of mRNA which is particularly imprecise if the cDNA is only partially hybridisable (that is, ~75% or less). We prepared cDNA complementary to mouse brain poly(A)$^+$mRNA which was hybridisable to 91–92% S_1 resistance. From hybridisation kinetics the complexity of the poly(A)$^+$mRNA was estimated to be 1.1×10^5 kilobases[10], a value which is similar to that obtained by the scDNA hybridisation method (Table 1). We conclude that the scDNA hybridisation method does not lead to gross overestimates of the complexity of RNA populations for reasons offered by Kiper.

WILLIAM E. HAHN
JEFFREY VAN NESS
IAN H. MAXWELL

Department of Anatomy,
University of Colorado,
School of Medicine,
Denver, Colorado 80262

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HYBRIDISATION of labelled single copy DNA with an excess of RNA is an important procedure for estimating the number of genes expressed in cells[1]. Recently, Kiper[2] asserted that gene number values in plants obtained by using this method are routinely significant overestimates. To support his premise, Kiper cited our measurements of the number of genes expressed in the tobacco leaf[3]. We contend that our original interpretation of the tobacco data[3] is theoretically sound and that the re-interpretation by Kiper[2] is based on specious arguments.

Kiper's first argument maintained that a large fraction of interspersed single copy DNA (scDNA) in plant genomes consisted of $\leq$300-base pair, unexpressed sequences. During the kinetic isolation of scDNA, these short, interspersed, unique sequences would be selectively lost due to their close linkage with repetitive sequence elements. The effective result (as Kiper maintains), therefore, would be an enrichment for expressed sequences in the single copy preparations used for DNA/RNA hybridisation experiments. Most plant genomes investigated to date, however, do not possess very short interspersed single copy sequences[4]. For example, the vast majority of interspersed single copy sequences in tobacco[5], soybean[6–8], cotton[9], corn (S. Hake and V. Walbot, unpublished), wheat[10], and mungbean[11] exceed 1,000 base pairs. Only in pea[12] and perhaps rye[13] does there seem to be a fraction of short scDNA. Direct electron microscopic observations have shown that the minimum number average lengths of interspersed single copy sequences in soybean[7] and cotton[9] are 1,300 and 2,900 base pairs respectively. Few short (<500 base pairs) single copy sequences were visualised (<5%). As the fragment length (200–300 bases) of scDNA used in gene expression studies is much less than that of the interspersed unique DNA, it is unlikely that a separate complexity class of unexpressed, 'cryptic', single copy sequences will be removed during scDNA isolation. The scDNA mass that is lost (due to short period interspersion effects) will simply be that which is at the termini of moderately long scDNA regions. Such regions have been demonstrated to code for mRNA[14–16]. We argue, therefore, that short plant scDNA preparations of the type used in RNA-excess/scDNA hybridisation experiments will not be enriched for expressed sequences or any other sequences as Kiper asserts.

Kiper's second argument was based on recent observations which have demonstrated that several animal genes are interrupted by non-coding sequences (intervening sequences)[17]. He contends, correctly, that if intervening sequences are present in plant genomes, they could potentially lead to an overestimate of gene number when DNA/RNA hybrids are assayed by binding to hydroxyapatite. However, as shown in Table 2 this is not the case, as similar gene number values were obtained with the scDNA method

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Table 1 Complexity of RNA from mouse brain assayed by HAP or S_1 nuclease

RNA	Method of assay*	scDNA as hybrid (%)	Complexity‡ (kilobases)
Poly(A)$^+$mRNA, brain	S_1 nuclease	3.9	1.25×10^5
Poly(A)$^+$mRNA, brain	HAP	4.1	1.33×10^5
Poly(A)$^+$mRNA, brain	HAP	3.6†	1.17×10^5
Nuclear, brain	S_1 nuclease	18	5.85×10^5
Nuclear, brain	HAP	19	6.17×10^5
Nuclear, brain	HAP	19.9†	6.46×10^5

* Assays were as previously described[8,9] with slight modifications.

† Previously reported value[8].

‡ Complexity estimate calculated on the basis that the single copy fraction of the mouse haploid genome is 3.25×10^9 nucleotides.

using both S_1 nuclease (which would eliminate non-coding sequence effects) and HAP assays. These data demonstrate that the presence or absence of intervening sequences has an insignificant effect on gene number values obtained by using HAP analysis with short single copy tracers.

In support of his arguments Kiper compared values obtained from mRNA/cDNA hybridisation studies[18] with those derived from RNA/single copy analysis[1]. In the case of our data[3] Kiper noted 'strikingly different' estimates of the number of genes expressed in the tobacco leaf from the two procedures (12,000 against 27,000). In our original report we discussed the inaccuracies inherent in gene number values derived from cDNA hybridisation. We stated explicitly that the data were consistent with a range of 6,000 to 24,000 diverse gene transcripts (see Fig. 3 and Table 1 of Ref. 3) and were thus consistent with the value obtained from the single copy procedure (see Fig. 4 and Table 2 of Ref. 3). As we and others[19–22] have discussed, computer solutions of cDNA hybridisation results must be interpreted with circumspection. In the absence of other information (for example, hybridisation analysis of isolated rare message cDNA), the gene number values computed cannot be considered unique and are accurate to only within a factor of 2.

Even if there are only 12,000 diverse gene transcripts represented in the tobacco leaf poly(A)$^+$mRNA population, this value would still be compatible with 27,000 gene transcripts in total mRNA. Approximately 50–70% of leaf mRNA is non-adenylated[3,23]. Several laboratories[24–26] have demonstrated that poly(A)$^+$ and poly(A$^-$) mRNAs may contain distinct subsets of gene transcripts. For example, there are about 120,000 diverse gene transcripts present in the poly(A$^+$) mRNA of mouse brain cells compared with 220,000 in total mRNA[24,27]. Thus, a comparison of cDNA hybridisation data with scDNA hybridisation data cannot be used to support Kiper's contention that the latter method overestimates gene number.

Finally, we wish to point out that gene number values, as estimated by any procedure, cannot be considered absolute. They are limited by many factors, including the presence of undetectable rare mRNAs (<0.1 molecule per cell), the use of abundant mRNA sizes to calculate gene numbers[28], salt correction factors used in kinetic analysis (W.E.H. unpublished) and the precision in measuring very small proportions of hybridised DNA. At the present, however, single copy measurements produce the most precise and reliable estimates of gene numbers, even in higher plants.

ROBERT B. GOLDBERG
Department of Biology,
University of California,
Los Angeles, California 90024

WILLIAM E. TIMBERLAKE
Department of Biology,
Wayne State University,
Detroit, Michigan 48202

1. Galau, G. A., Britten, R. J. & Davidson, E. H. *Cell* **2,** 9 (1974).
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KIPER REPLIES—The first point stressed in my recent paper[1] was that in eukaryotic single copy DNA prepared for mRNA complexity measurements short unique sequences are absent and ends of longer unique sequences are under-represented while long unique DNA sequences will be over-represented. This is caused by sequence interspersion of different kinetic classes and random shearing. Thus taking the total nuclear unique DNA content as a basis for complexity calculations, gene number overestimates result if mRNAs are preferentially encoded for by longer unique sequences.

Concerning the sea urchin mRNA complexity measurements, the degree of over-representation could indeed be 1.5 as could the gene number overestimate: so, at a length of 450-nucleotide reassociation kinetics displayed a 50% unique DNA content[2]. Galau *et al.*, however, took a 75% content as the basis for their estimates[3], a value derived from the fraction of S_1-digestible C_0t 20 reassociated DNA[2].

The point questioned by Goldberg and Timberlake is whether genomes (especially of the plant kingdom) will contain gross amounts of short cryptic unique DNA. This, now, is certainly proved for pea DNA. Murray *et al.* demonstrated that the major class of single-copy sequences has a modal length of about 300 nucleotides (ref. 4) and that due to the intimate interspersion with repetitive DNA no true single copy tracer can be prepared[5]. Goldberg and Timberlake's contention is that in tobacco (and other plants) very few unique sequences are shorter than 500 base pairs and the average length of interspersed scDNA sequences is at least 1,400 nucleotides. Hence the scDNA preparation is a fair representation of the total scDNA complexity, cryptic DNA thus becoming negligible. Their data, however, seem to conflict with this view: so, in HAP renaturation kinetics analysis of a fragment length of 300 nucleotides 22–23% of the whole genome was included in the unique DNA fraction. This increased to 43% using S_1 nuclease (47% after some minor correction)[6]. Their mRNA complexity measurements were based on the complexity of a 42.5% scDNA fraction[7]. Now think of a population of interspersed unique DNA sequences averaging 1,400 nucleotides; then during reassociation analysis of sheared 300-nucleotide-long whole genomic DNA, 81% of this would be scored as unique DNA using HAP analysis (for formulae see ref. 1): that is a

Table 2 Estimates of gene number derived from the single copy procedure using hydroxyapatite and S_1 nuclease assays

Organism	Assay		Ref.
	HAP	S_1 nuclease	
Tobacco	27,000	24,000	3*
Achlya	2,000	3,200	29, 30
Mouse brain	100,000	120,000	24, 27

*J.C. Kamalay and R.B.G., unpublished.

23% increase would be expected, revealing the whole unique DNA content using S_1 nuclease. The 87% increase found in tobacco (taking the corrected value of 47% instead of 43% this amounts to a more than 100% increase) seems rational only at an average unique DNA fragment length of less than 600 nucleotides. As many sequences seem to be longer than 1,000 nucleotides this favours a broad class of rather short unique sequences behaving cryptically. Only if all these unique sequences—whether short, medium or long—code for mRNA to the same extent would it be valid to base gene number estimates of tobacco and other organisms on the total nuclear unique DNA complexity. However, this is unlikely considering what is now known about mRNA length and organisation of individual genes.

Hahn *et al.* argue that coding sequences are extensively represented in a repetitive sequence neighbouring unique DNA, which is, as I pointed out, under-represented in scDNA preparations; nevertheless it would still be valid to refer to the total unique DNA complexity. In support of this they cite the sea urchin experiment of Davidson *et al.*[8]. However, the mRNA mass–complexity equivalent postulated for this experiment is not backed by any results. In that experiment 77% of the mass of all mRNA seemed to be adjacent to interspersed repetitive sequences; but, as only a total of 8 ± 4% of all mRNA mass represented almost the whole complexity[3], the results can equally be interpreted as meaning that only a few (abundant) mRNAs were adjacent to repetitive DNA. Furthermore, the HAP assay was used, and in view of recent insights into gene interruption this technique could have measured minor coding portions of genes adjacent to repetitive elements. The main argument, however, is that the last fractionation in purification of the repeat contiguous DNA excluded all unique DNA behaving cryptically in reassociation kinetics from the pool which was allowed to hybridise with the mRNA population. Therefore, this experiment is irrelevant to the question.

The finding of Hahn *et al.* that in mouse only 10% of HAP-assayed repetitive DNA is digested by S_1 nuclease is in contrast with other findings, for example, in the sea urchin[2] and tobacco[6] where 50% and 43%, respectively were digestible. Thus the mouse experiments do not seem to be representative and favour minimal interspersion between different kinetic classes in the mouse genome.

Concerning intervening sequences—the second point of my paper—their general occurrence will have to be deduced by further research. A close look at the organisation of individual genes, as carried out for the globin genes[9] or the ovalbumin gene[10] and the ovomucoid gene[11] and lysozyme[12], has revealed the existence of rather short gene domains separated by intervening sequences of about equal length or longer. If the intervening sequences are unique in the genome too, as shown for the globin[13] and the ovalbumin gene[14], that is if genes are located in long stretches of unique DNA, a large fraction of the introns will be scored as coding complexity in the scDNA saturation experiments described. Only if the length of the scDNA preparation is much smaller than the exons would interference by intervening sequences be unimportant. However, most of the coding domains known today are even shorter than the scDNA preparations routinely used in complexity measurements[9–12]. The data compiled by Goldberg, Timberlake, Hahn and coworkers in their critiques seem to contradict the argument. Apparently, however, to solve the problem it will be necessary to check HAP-isolated hybrids for their content of non-coding single-stranded DNA loops.

Previous interspersion analysis of many genomes has demonstrated that sea urchin DNA organisation is not typical of eukaryotic genomes as postulated by Hahn *et al.*; nor is any other sequence organisation pattern. Eukaryotic genomes differ largely in their content of repetitive DNA and their interspersion patterns. Long period interspersion of unique DNA was found to be predominant in birds[15], fungi[16] and insects[17]. Higher plants show varying interspersion patterns with unique DNA lengths of 300 nucleotides being predominant in pea[5] while *Xenopus*-like patterns are predominant in tobacco[6]. Short unique sequences can be separated by short repetitive sequences (300–400-nucleotide fragments in pea[6]), repetitive sequences 1,250 base pairs long (as in cotton[18]) or by repetitive sequences thousands of base pairs long, as in the genome of pearl millet[19]. Higher plants with low haploid genome size contain half of their scDNA in stretches a few thousand base pairs long[19,20]. Most other plant and animal genomes also contain long unique DNA sequences. So, the question remains whether coding sequences are typically located within long stretches of unique DNA.

However, genome and gene organisation complicate interpretation of DNA saturation experiments. Furthermore, many considerations lead to the conclusion that scDNA saturation experiments will routinely overestimate numbers of active genes: over-representation of coding sequences in the unique DNA preparation, split genes, few-copy DNA taken for scDNA, gene duplication as in the human globin genes, cross hybridisation of certain gene domains[21], and scoring of unique gene flanking sequences as coding. So, many detailed answers have to be given before the results can be interpreted with confidence. This will be a more severe problem with genomes containing large amounts of repetitive DNA and extensive interspersion with unique DNA, as is typical in higher plants.

MANUEL KIPER

Institut für Botanik,
Universität Hannover,
3000 Hannover, FRG

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Aminostratigraphy of Pleistocene and Holocene deposits

RECENT work on amino acid ratios for sites in the UK is a major step forward in the research of the Pleistocene and early Holocene periods[1,2]. Archaeological work, coupled with environmental studies, can be valuable in supporting amino acid research or in pinpointing possible discrepancies and problems. Miller *et al.*[1] have compared the Purfleet and Swanscombe Pleistocene sites, pointing out that the mean D-alloisoleucene: L-isoleucene ratio for Purfleet is 0.35 while for Swanscombe the ratio is 0.30. At Purfleet, as at Swanscombe, there are three main gravels separated by sands and silts, and the artefacts from Purfleet are comparable with those of the Middle Gravels from Swanscombe[3]. The shell samples used from

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Swanscombe came from the Middle Gravels but those from Purfleet came from a shell band which is ~1 m or more below the main or Middle Gravel at that site, immediately above a coarse sandy orange-brown gravel, referred to as Gravel 3. Very few artefacts came from this Lower Gravel, but some of Clactonian aspects were present. It can, therefore, be suggested that the whole series of deposits at Purfleet are comparable to those at Swanscombe. As the amino acid ratios from Purfleet refer to deposits which are stratigraphically somewhat older than the Middle Gravel at Swanscombe, the discrepancies in the ratios can possibly be explained on this basis as dating slightly different phases of the same interglacial.

Andrews, Bowen and Kidson[2] pointed out that the Portland Raised Beach and Burtle Beds have produced ratios which do not agree exactly with those of Middle Hope and Minchin Hole, all frequently assigned to the last interglacial. There may be several explanations for these discrepancies. Attention has often been drawn to the fact that there are pronounced altimetric differences between the Beach deposits in various parts of the Bill area of Portland, and it has recently been pointed out that there may be at least two quite distinct raised beaches on the Isle[4]. Here again, different phases of the same interglacial may be involved, indicating minor advances and regressions of the sea.

On the other hand, other environmental factors could possibly be involved, as suggested by the ratios of the Holocene sample from Portland. This sample came from the Mesolithic habitation site of Culver Well, with a very large shell-midden with uncalibrated radiocarbon dates of 5200±135 BC (BM-473) and 5151±97 BC (BM-960); a weighted average of three samples of burnt limestone from a hearth has given a thermoluminescence date of 5400 BC±640 (OxTL 501 b, m)[5]. The stratigraphical evidence and archaeological finds from the midden clearly indicate a dating within the Mesolithic period, falling within the Atlantic climatic zone. The other Holocene sample came from Skara Brae in the Orkneys, a site known from the archaeological evidence to belong to the late Neolithic period associated with pottery of the Rinyo type, that is this site is at least 3,000 yr younger than Portland (or less than half the age of the Mesolithic site). Yet both sites have produced D-allo: L-iso (combined) ratios of 0.04, indicating that results from one or the other, or perhaps even both, the Holocene control samples are unreliable. This too could possibly be explained by different environmental or economic circumstances relating to the sites, during the relevant prehistoric periods and after, affecting the epimerisation reaction of the samples. Or it may be that the sensitivities of the techniques are not yet so advanced that differences of only a few thousand years can be registered.

These discrepancies do not, however, minimise the great value of the work being done on amino acid reactions as potential chronological indices.

SUSANN PALMER
London Borough of Bromley Museum,
Priory, Orpington, Kent, UK

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MILLER *ET AL.* REPLY—Our *Corbicula* from Swanscombe are not from, but merely correlated with, the Middle Gravels there. If the correlation is correct, then the absence of *Corbicula* in the Swanscombe Lower Loam and Lower Gravel, at an elevation intermediate between the Middle Gravels and Grays (with similar ratios), suggests that the Lower Loam and Lower Gravel may be from an older interglacial. The *Corbicula* at Purfleet could be from early in the Middle Gravels interglacial, or from another interglacial, intermediate in age between the Middle Gravels and Lower Loam–Lower Gravel ones.

At current temperatures in Britain, amino acid epimerisation occurs slowly; hence, as Palmer infers, we cannot detect as yet differences of a few thousands of years. This does not mean that such discrimination is not possible. Instrumentation is improving rapidly, and there are several different reactions that may give the resolution required.

Ours[1,2] are preliminary analyses: our papers have demonstrated that amino stratigraphy will provide a useful time-frame for UK Pleistocene studies. To that end, we would like to say that we are very willing to cooperate with UK researchers on problems where this technique may be applicable.

J. T. ANDREWS
J. T. HOLLIN
G. H. MILLER
INSTAAR and Geological Sciences,
University of Colorado, Boulder,
Boulder, Colorado 80309

1. Miller, G. H., Hollin, J. T. & Andrews, J. T. *Nature* **281**, 539–543 (1979).
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Asymmetry in relatedness: who is related to whom?

FLESNESS[1] has brought out the interesting fact that relatedness between diploids can be asymmetric, as it is between the sexes of male-haploids[2]. His note, however, contains a conceptual difficulty. This concerns the directionality of relatedness in his otherwise valuable definition. Because relatedness calculations are usually made in order to determine the (theoretical) optimum course of action of a potential altruist *vis-à-vis* a particular recipient, we suggest that the quantity implied in the phrasing 'relatedness of recipient to donor' should be the genetic similarity of the recipient to the donor, but under Flesness's definition it is the genetic similarity of the donor to the recipient. If weighing up (in terms of relationship) the benefits of being altruistic towards someone, you should care about the proportion of your genes that is present in him, and not the portion of his genes present in you. We therefore redefine Flesness's coefficient:

$$R_{B(A)} = \text{relatedness of B to A}$$

$$= \frac{\text{(mean identity by descent of A's genes with B's gametes)}}{\text{(mean identity by descent of A's genes with A's gametes)}}$$

This directionality is also the same as that adopted by Hamilton[3] in discussing essentially the same parameter. As a consequence, the values given in Flesness's table should be reversed.

$R_{B(A)}$ (as defined above) has the same meaning as the 'pedigree coefficient of relatedness', $G_{B(A)}$ (ref. 2, and R. Frankham, in preparation), and is also similar to the 'regression coefficient of relatedness', $b_{B(A)}$ (ref. 3) (with the subscript order reversed here to conform with standard regression notation). We suggest that $G_{B(A)}$ be retained for use as the central measurement of relatedness in pedigrees (as $R_{B(A)}$ is more easily confused with Wright's[4] 'coefficient of relationship', r), and that Hamilton's coefficient be retained for estimates of relatedness derived by regression analysis of populations (P.P. and R.H.C., in preparation).

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R. H. CROZIER*
P. PAMILO†
School of Zoology,
University of New South Wales
Kensington, New South Wales,
Australia 2033

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* To whom reprint requests should be sent.
† Present address: Department of Genetics, University of Helsinki, Helsinki, Finland.

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Enigmatic variations

A.B. Pittock

Sun, Weather, and Climate. By J.R. Herman and R.A. Goldberg. Pp.360. (National Aeronautics and Space Administration: Washington, DC, 1979.)

THIS well produced paperback addresses the question as to what influence solar variations have on weather and climate. This is a highly controversial and (as I know from personal experience) an emotional subject.

So let me, at the outset, lay my own established position on the line. Quoting from my published review "... it seems that despite a massive literature on the subject, there is at present little or no convincing evidence of statistically significant or practically useful correlations between sunspot cycles and the weather or climate." (*Rev. Geophys. Space Phys.*, **16**, 400; 1978). At the time I wrote that (late 1977), swayed particularly by the critical examination by C.O. Hines and I. Halevy (*J. atmos. Sci.*, **34**, 382; 1977) of the work of J.M. Wilcox *et al* (*J. atmos. Sci.*, **33**,1113; 1976), I conceded that there were "... apparently significant short-term correlations between meteorological indices and the passage of solar magnetic sector boundaries. . .". Now that I have read R.G. Williams and E.J. Gerety (*Nature*, **275**, 200; 1978) and R. Shapiro (*J. atmos. Sci.*, **36**, 1105; 1979) on that particular subject I am not so convinced by the short-period effects either.

Contrast these rather negative comments with the more positive upbeat approach and conclusions of Herman and Goldberg. In the preface (page v) we read: "Even though the reported results have sometimes been confused, disjointed, and contradictory, there has emerged a growing belief that there are connections between changes on the Sun and changes in the lower atmosphere". In the penultimate chapter (page 274) entitled "Recapitulation of Sun-Weather Relationships" we read that "In summary, the correlations between solar activity and meterological responses are highly suggestive and require further investigation. To take an agnostic point of view, these relationships have not been proven or disproven and therefore require further attention.".

What accounts for this apparently profound difference in our assessments of essentially the same evidence? I wish the philosophers of science would address themselves to this particular controversy because I suspect that in the literature on the solar weather/climate relationships they will find their best possible case study of objectivity versus subjectivity, and of the conscious or unconscious influence of vested interests and disciplinary prejudices. It is for instance almost axiomatic in this controversy that meterologists and climatologists will applaud my 'critical' approach, while ionospheric and solar physicists will prefer Herman and Goldberg's more optimistic or 'agnostic' approach.

The cynics will put this down to blatant self-interest, each group seeking to justify its own research interests and even secure funding for its more expensive projects. But I believe it runs deeper and is more subtle than that. Whatever the motivation, the disbelievers and skeptics demand a degree of statistical rigour and level of proof which the believers feel to be unnecessary or destructive of a potentially important physical and practical insight into nature. The protagonists can point to the well established proven influence of solar variations on the ionosphere and mesosphere and suggest that such influence must extend down into the meteorologically active regions. The presumption is that such effects exist in the lower atmosphere and the problem lies in establishing the magnitude and precise nature and mechanisms of such effects.

What we are seeing in this controversy is thus a reversal of the onus of proof or of the null hypothesis from that which is usual in science. The protagonists wish to use as their null hypothesis the proposition that solar effects do exist, and are thus satisfied (at least for now) with a situation in which this has not been disproven beyond reasonable doubt. The opposite null hypothesis, that no effect exists until it is proven to exist beyond a reasonable doubt is the traditional hypothesis which safeguards the purity of established scientific theory. In the present state of the solar weather/climate controversy these two positions are poles apart in terms of the level of proof or rigour applied to the available data.

To take a couple of specific (if perhaps extreme) examples from Herman and Goldberg's book, let us look at their treatment of two of the more spectacular and well known claims of a significant effect of solar cycles on climate.

Herman and Goldberg report concerning thunderstorm frequency (page 142) "The strongest correlation [with sunspot numbers] (about + 0.9) is found in Siberia and has been consistently reported as such for many years (Septer, 1926; Shaw, 1928; Brooks, 1934). Likhter reported the same value again in 1968 (Markson, 1971)." [*Pure Appl. Geophys.*, **84**, 161; 1971]. They then go on to discuss these results, stating that the "original work on Siberian thunderstorms was apparently done by Septer" and noting that Brooks used Septer's data, but not making clear that Shaw also used Septer's data and that Likhter *et al.* did not find such a strong relationship using more recent data. Markson's reference to Likhter *et al.* (1968) is to an unpublished conference paper, but a paper with the same authors and title is Likhter, Ia.I, Kolokolov, V.P. and Kleimenova, E.P.; *Trudy, Glavnaia Geofiz. Abs.* No.242, 104; 1969. They used IGY–IQSY data and found a more "complex relationship" than that reported by Brooks. Instead of four independent and consistent results we thus have only one, which is not so impressive. Herman and Goldberg compound this inflated impression of Septer's results by failing to note that in fact later surveys by H.J. Fischer and R. Mühleisen (*Meteorol. Rundsch.*, **25**, 6; 1972), and Z.P. Kleimenova (*Meteorol. Gid rol.*, No.8, 64; 1967) using more recent data fail to find significant positive correlations between thunderstorm frequency in Siberia and sunspot numbers. Indeed Kleimenova reveals that she could not find records from as many stations as Septer claimed to have used. Kleimenova then attempted to verify Septer's result with all the available data for the same period and concluded that "the results obtained by Septer are not in accord with fact. . .". How unfortunate that one of the most spectacular and oft repeated claims in the literature rests on data which can no longer be found! What weight can we give to this claim?

The second example is the authors' treatment of the claims by Xanthakis for large and significant positive or negative correlations between zonal indices of precipitation and sunspot activity. Herman and Goldberg here faithfully report the reversals of sign in correlation coefficients between zones and between epochs in the same zones and also note the inconsistencies of J. Xanthakis' results (in *Solar*

Activity and Related Interplanetary and Terrestrial Phenomena; Springer: New York; 1973) with those of E.S. Gerety *et al.* (*J. atmos. Sci.,* **34**, 673; 1977) and M. Dehsara and K. Cheak (*Arch. Met. Geoph. Biokl.,* Ser. B, **18**, 253; 1970). But they seek to explain these, not by a critical examination of the conclusions of, methodology, and data sets used by Xanthakis, but by appealing to a more complex hypothesis allowing more degrees of freedom. Thus on page 130 they say "If the pattern of the global distribution of excess rainfall . . . undergoes secular changes due to long-term variations in extraterrestrial or even terrestrial influences, averages over wide geographic expanses could very well exhibit correlation reversals with sunspot number . . . Analysis along (these) lines might reconcile some of the discrepancies . . .".

This is a common argument used by the protagonists of solar effects but in the absence of specific theories as to how things ought to change in time or space it simply allows infinite room to manoeuvre, 'explaining' all inconsistencies and proving nothing. It is to the authors' credit that they make this argument fairly explicit in a section headed "Correlation Reversals and Failures", but it is nevertheless a dangerous argument since it removes the incentive towards a truly critical examination of the claims in the literature. As if to prove my point, subsequent admissions by J. Xanthakis (*Nature,* **280**, 254; 1979) that he in fact selected his data *a posteriori* have now clearly invalidated the significance of his claimed results.

Similar criticisms can be made of the authors' rather uncritical acceptance of many other contradictory claims in the literature, for example in their discussions of much of King's work, including King's treatment of rainfall at Fortaleza (pages 119-120) and of Bowen's claims for Australian rainfall (pages 88 *et seq*).

So far my comments have been rather negative. However I want to pay tribute to Herman and Goldberg for several important things. One is the comprehensive (if not complete) nature of their survey and its clear presentation. In particular the excellent indexing greatly enhances the value of the book. Their highlighting of some of the important inconsistencies and breakdowns in the reported correlations is another big plus.

Perhaps the greatest virtue of the book is its emphasis on the need to develop and test physical theories without which the study of solar-weather/climate relationships will never emerge from its present morass. This emphasis will be found in the authors' very first sentence (Preface, page v) and is substantiated in a chapter devoted to possible "Physical Processes and Mechanisms" (chapter 6) and in the final chapter "Guidelines for Experiments" (chapter 8). Inevitably, coming from people deeply involved in research in relevant areas, this emphasis seems to be rather self-serving. Nevertheless it is a necessary and correct general emphasis, whatever we may think of their particular suggestions and their too ready acceptance of apparently relevant but dubious claims in the literature.

In summary then, I find Herman and Goldberg's book to be comprehensive (although not perfectly so) but regrettably uncritical in its approach to the literature. It has a desirable emphasis on the need for physical theories and explanations, and on attempting to isolate critical experiments. It also draws together information concerning a wide range of possibly relevant phenomena and processes. Although the case for rejecting the null hypothesis that there are no significant effects has not been proven by this book, there nevertheless remains a case for supporting further research directed with physical insight at the hypothesis that there just might be significant effects. We might at least usefully attempt to see how far down such effects penetrate into the atmosphere and to set upper limits on their magnitude in the troposphere. Read with healthy skepticism and critical discernment, Herman and Goldberg's book makes a useful contribution to such an effort. ☐

A.B. Pittock is a Principal Research Scientist with the CSIRO Division of Atmospheric Physics, Mordialloc, Australia.

Kinetoplastid biology

J.R. Baker

Biology of the Kinetoplastida. Vol. 2. Edited by W.H.R. Lumsden and D.A. Evans. Pp.738. (Academic: London, New York and San Francisco, 1979.) £45; $94.

VOLUME 1 of this two-part work is an invaluable reference on trypanosomes and leishmanias, serving as an 'in-filler' of gaps in other standard works as well as presenting new developments in better-known fields. The present volume seems certain to follow suit. Its net is cast wider than its predecessor's, but the emphasis of its fifteen chapters is on parasites of man and domestic animals.

J.J. Shaw and R. Lainson review the ever-changing scene of leishmanial taxonomy and epidemiology and introduce three subgeneric "sections". R. Killick-Kendrick fully discusses *Leishmania* in its vector; and V.P. Sergiev describes the epidemiology of leishmaniasis, now mainly zoonotic, in USSR. M.A. Miles discusses biochemical and immunological heterogeneity of *Trypanosoma cruzi* and the problems arising therefrom; and its pathogenesis and biochemistry are reviewed respectively by W.L. Tafuri and by W.E. Gutteridge and G.W. Rogerson. W.E. Ormerod considers old and new concepts of the life cycle of *T. brucei* in mammals and heretically suggests that it is not the "stumpy" form which infects tsetse, and that a special "tissue phase" exists. W.J. Herbert and D. Parratt stimulatingly discuss virulence ("the capacity . . . to damage . . . its host") and heterophile antibodies in (almost exclusively African) trypanosomiasis, speculating on their significance.

Other chapters deal with kinetoplastids of arthropods (F.G. Wallace), lizards (V.C.L.C. Wilson and B.A. Southgate), fish (J. Lom), including lists of 149 alleged species of *Trypanosoma* and 33 of *Trypanoplasma*, and rodents (G.A.T. Targett and P. Viens), with emphasis on recent immunological work (much of it their own). Two general chapters include one on nutrition (S.H. Hutner, C.J. Bacchi and H. Baker) and one on characterisation, nomenclature and maintenance (W.H.R. Lumsden and D. Ketteridge).

Inevitably, perhaps, the choice of topics and their arrangement seems somewhat random; some deal with well known groups, presenting new information and syntheses, while others deal with parasites less often reviewed. Both groups are valuable, all contributions being authoritative, concise, comprehensive and comprehensible. Standards of editing and production are high — as, alas, is the price. There is a good general index (though the alphabetisation under the massive subheading *"Trypanosoma"* takes some getting used to), but no author index. Altogether, a volume to be recommended and either bought or coveted by all teachers or researchers of the kinetoplastids: it is sad that further volumes are not planned "at present". ☐

J.R. Baker is at the MRC Biochemical Parasitology Unit, Molteno Institute, Cambridge, UK.

- The second edition of *The Dynamics of the Upper Ocean*, by O.M. Phillips (for review, *see Nature,* **272**, 194; 1978), has been published by Cambridge University Press in paperback at £7.95.

- In the review of *Image Analysis, Enhancement and Interpretation,* by D.L. Missell (*Nature,* **282**, 885; 1979), the prices were incorrectly quoted, thus making the final criticisms of the reviewer unfounded. These should have read: Hardback $60, Dfl.135; paperback $31.75, Dfl.65.

- In the review of *Scientific Models and Man* (*Nature,* **283**, 113; 1979), the name of one of the lecturers was incorrectly quoted. This should have been: Donald Michie.

Planetary harmony

David W. Hughes

The Harmony of the World: A Realization for the Ear of Johannes Kepler's Astronomical Data from Harmonices Mundi, 1619. Gramophone record. By John Rodgers and Willie Ruff. (Sky Publishing: Cambridge, Massachusetts, 1979.) $11.50 (including postage and packing).

KEPLER, apart from being one of the greatest, is also one of the more endearing astronomers because of his habit of including in his books a detailed account of the blind alleys he went along as well as the paths of success. He was convinced that the ways in which knowledge is acquired are as important as the knowledge itself. It's a pity more people don't think the same today.

One of Kepler's main goals in life was the discovery of the mathematical rules underlying the progression of planetary orbital sizes, shapes and periodicities. A blind alley on the journey to this goal was the hypothesis of planetary harmony — the mathematical music of the spheres. To quote from Kepler's book *Harmonices Mundi:* "The heavenly motions are nothing but a continuous song for several voices, to be perceived by the intellect, not by the ear; a music which, through discordant tensions, through syncopations and cadenzas as it were, progresses toward certain predesigned six-voiced cadences, and thereby sets landmarks in the immeasurable flow of time".

Each planet produces a tone proportional to its angular velocity as seen from the Sun and a tonal range which depends on the orbital eccentricity. The frequency of the note is a function of the heliocentric distances. Figure 1 shows the notes that Kepler thought the planets played.

Now Kepler might have been content to perceive the heavenly chords 'by the intellect' but I have always wanted to hear them in reality and to listen to how they change as the planets orbit the Sun. Thanks to Professor Ruff of the School of Music, Yale, and Professor Rodgers of the Geology Department of the same university, this is now possible. The first 'fixed point' of their realisation is the mean frequency of the Earth, which they set at 800 Hz — very close to the g″ (a musical expression for the g note about an octave and a half above middle c) shown in Figure 1. The second fixed point is the orbital period of Pluto (248yr) which they musically set to be 20 min 42 s. Ruff and Rodgers have updated Kepler by introducing Uranus, Neptune and Pluto. As these planets have such low frequencies they have been simulated by a series of rhythmic beats — Uranus around 10 to the second, and so on. The long playing record covers the harmony of the spheres between 27 December 1571 (Kepler's birthday) and 1835. The record also includes introductions to the planetary sounds one by one. This sequence would have been much more useful if there had been a voice announcement introducing each planet.

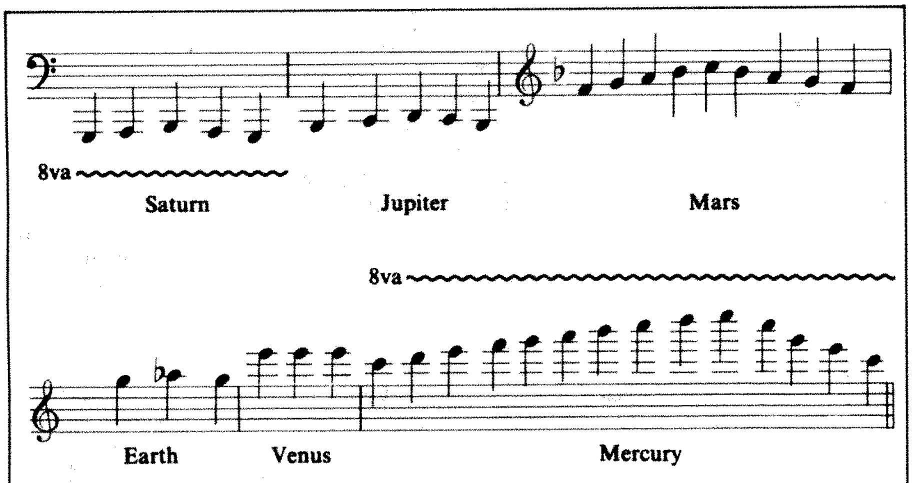

Figure 1 The 'notes' played by the planets.

Scientifically the record has considerable curiosity value, and the inclusion of copious sleeve notes and a copy of an explanatory paper by Rodgers and Ruff *(American Scientist,* **67**, 286-292; 1979) makes it all the more useful.

Musically the sounds have been computer generated using an IBM 360/91, and I am in deeper water. My scant musical knowledge comes from struggling through to Grade 8 piano and my musical appreciation falls into the "I know what I like" category. Bearing these facts in mind I conclude that this record put on near the end of a party would be guaranteed to clear the room in minutes flat. ☐

David W. Hughes is Lecturer in Physics and Astronomy at the University of Sheffield, UK.

Statistics in particle physics

H. Muirhead

Probability and Statistics in Particle Physics. By A.G. Frodesen, O. Skjeggestad and H. Tofte. Pp.501. (Universitetsforlaget: Bergen, Oslo and Tromso; Columbia University Press: New York, and Guildford, UK, 1979.) NKr.150; $30.

WHEN I saw the name of Skjeggestad among the authors of this book, I immediately recalled a set of lectures given by him on phase space at a CERN post-graduate school in 1964. These lectures were published as a CERN yellow report and have been part of the staple diet of research students ever since. I can see this book being used in much the same way, but not only by research students.

This book is no 'fly-by-night' progress report. It contains much solid, and at times, difficult material presented in a readable way. Although it is aimed at particle physicists the clarity of presentation should give it wider appeal. The many examples are excellent and treat real situations in particle physics. There was for me one notable omission, however, namely the treatment of the significance of a bump ('Breit-Wigner') on a background. If the bump is big and narrow and the background flat then there is no problem. If the bump is not so big and coincides with the peak in the background and one is not completely sure about the shape of the background, then the procedure becomes tricky to put it mildly.

One further thing I would like to have seen. A general warning (almost like on cigarette packages) about using common-sense, as the treatment of errors can drive one mad. I have too often seen computer output from students containing measured quantities with nonsensical errors or χ^2, not because the error treatment was wrong but because it had not been applied in a sensible way. One finds hints of this in the book — on page 289, for example, in discussing "pulls" the reader is advised to throw out a nonsense point from the data if he gets a bad value for χ. However, I would like to have seen a more general warning given, possibly in the preface. In reading the preface I noted that the authors paid tribute to Kendall and Stuart for their *The Advanced Theory of Statistics* (Charles Griffin: London, 1963) in three volumes, which they constantly consulted. It was also my staple stand-by 30 years ago; then it was in two volumes and had one author. I wish the present authors good fortune. ☐

H. Muirhead is Reader in Physics at the University of Liverpool, UK.

Facets of the fern

R.M. Lloyd

The Experimental Biology of Ferns. Edited by A.F. Dyer. Pp.657. (Academic: London, New York and San Francisco, 1979.) £37.50; $79.

FERNS exhibit all basic phenomena of the life-cycles of vascular plants in a form which is exceptionally suitable for experimental investigation. In spite of their geological age, the group as a whole is in an active state of evolution. Although numerically small relative to the rest of the world's flora, their contribution to our understanding of the physiology of differentiation and development, genetic systems and evolution, cannot be underestimated. This multi-authored book is an attempt to put together in one volume an overview of the ultrastructural, biochemical, physiological and genetical research which has taken place during the past 40 years in experimental pteridology. All significant facets of the fern life-cycle are covered, from meiosis and spore initiation, through gametophyte morphogenesis, gametangial initiation, gametogenesis and the influence of antheridiogens, to sporophyte development, apogamy, biosystematics and experimental ecology. The book is intended to make more biologists aware of the experimental research already completed in ferns as well as their potential for further highly productive research. To facilitate this, all of the authors have quite exceptionally circumscribed the available literature in their respective fields and have stressed not only those aspects which are in need of further work but also the currently prevalent controversial hypotheses.

Contributions of particular note are those by Peter Bell, who discusses the fern life-cycle in the conceptual framework of vascular life-cycles as a whole and presents the case for the cytological control of gametophyte sex as well as the evidence pertaining to the expression of gametophytic and sporophytic genes in spores and zygotes; Ed Klekowski, who summarises much of the evidence in support of his controversial theories on homoeologous chromosome pairing and the genetic system; J.M. Pettitt, who details our relatively meagre knowledge of spore wall structure, morphogenesis and cytochemistry; and Richard White, who effectively perpetuates the opposing arguments on the extent of developmental activity in the shoot and root apical systems. Over half of the text is devoted to research on the gametophyte generation, reflecting accurately the heavy emphasis it has received due, no doubt, to its ease of culture and manipulation. In contrast, modern ecological work in ferns is severely limited and the contribution by Chris Page adamantly exposes the critical need for further work in this area.

As this volume is technical and an excellent literature summary and as it emphasises the current sophistication and problems of experimental pteridology, it will be an important reference book for years, if not decades, to come. I, for one, am extremely pleased that it has appeared.

R.M. Lloyd is Associate Professor of Botany at Ohio University, Athens, Ohio, and is Vice-President of the American Fern Society.

Basic statistical mechanics

P. T. Landsberg

Convexity in the Theory of Lattice Gases. By R. Israel. Pp.167. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) £10.40.

BY developing a series of theorems of considerable abstractness and generality, the author links together the statistical thermodynamics of lattice gases (for example using models) and the theory of convex domains. The connection between the convexity and homogeneity of thermodynamic functions is considered (a) in a finite system and (b) in the thermodynamic limit when the number of particles n and the volume v of the system both tend to infinity in such a way as to keep their ratio constant.

This book is clearly essential reading for those interested in the modern discussion of basic statistical mechanics. It requires familiarity with the technical mathematics developed by Ruelle and colleagues. Both the concept of Hausdorff dimension and Banach space are here needed for a discussion of the Gibbs phase rule, which is treated in the sixth and last chapter of the book. New proofs concerning the existence of phase transitions are given in chapter five, by means of a modification of a theorem in pure mathematics about convex sets, which was given in 1963 by E. E. Bishop and R. R. Phelps.

To extract the physical significance of the results beyond the rather general indication given above is difficult, even if adequate space were available in this review. The job of obtaining an intuitive understanding of these detailed results is eased by the masterful Introduction which is provided by A. S. Wightman in 81 pages. Even so, much 'vulgarisation' will have to be done before this topic will find its way into the textbooks. □

P. T. Landsberg is Professor of Mathematics at the University of Southampton, UK.

Quantitative electron microscopy

V.E. Cosslett

Introduction to Analytical Electron Microscopy. J.J. Hren, J.I. Goldstein and D.C. Joy. Pp. 601. (Plenum: New York and London, 1979.)

THE history of light optical microscopy has shown a steady progression in the use of methods for obtaining quantitative information about a specimen. Modern high-power microscopes permit measurements of absorption, refractive index, birefringence and so on, as well as morphometry. It is not surprising, therefore, that electron microscopy is following the same trend, but with greater acceleration. During the past 25 years a whole range of *In situ* analytical techniques have been developed, from crystal structure determination by microdiffraction to localised chemical analysis by X-ray excitation and energy-loss spectrometry.

The present volume presents the first comprehensive attempt to bring this battery of research tools to the attention of electron microscopists. It comprises the lectures at a "Workshop" with the same title which was part of the annual meeting of the Electron Microscopy Society of America in August 1979. The editors succeeded in persuading the 22 co-authors to provide their manuscripts in time for the book to be printed and handed to participants when they arrived in San Antonio. Any editor of a collective work will appreciate the magnitude of this achievement, partly dependent perhaps on the use of a word processor.

We thus have available a complete review of the subject, starting with basic chapters on image formation and the electron optics of the microscope, through the instrumentation and application of the various analytical techniques, to discussion of radiation damage effects and the computer simulation of images. All the authors are expert in their particular fields, so that the numerous references are well selected and up-to-date. As acknowledged by the editors in the preface, such an enterprise undertaken at such a speed has inevitably resulted in a fair number of minor errors both technical and typographical. In view of the value of the product of their labours, however, we may accept the plea that these are "tolerable imperfections under the circumstances", and wish them the opportunity of a second edition in which they can be corrected. □

V.E. Cosslett is Emeritus Reader in Electron Physics in the Cavendish Laboratory, Cambridge, UK.

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A dearth of new drugs

THE Californian court case in which Mrs Betty Mekdeci is trying to establish that *Bendectin*, an anti-nausea drug manufactured by Richardson Merrell, was responsible for the deformities with which her son was born, can hardly have come at a worse time for those who thought they could at last see some light at the end of the tunnel of regulations in which the pharmaceutical industry has found itself since the thalidomide tragedy of 1961. Whatever the outcome of the case against *Bendectin* the blaze of publicity surrounding it is bound to make this a less than opportune moment for the political turn-about on regulations that seemed to be imminent, at least in the UK. Therefore it is important that the *Bendectin* case is used to illuminate rather than to obscure the arguments for and against relaxing regulations.

The pharmaceutical industry is prone to claim that were the regulations of the American Food and Drugs Administration to apply worldwide, no new drug would ever reach the market. Although that is hyperbole, it is true that regulations are more restrictive in the US than even in the UK which has the stiffest regulations in the European Economic Community. It is also true that the figures underlying the claims are dramatic. Compared to 20 years ago, it now takes about three times as long from the discovery to the marketing of a new drug. The demands for drug testing before marketing have risen enormously, some would say 100-fold. The number of new drugs that reach the market each year has been halved. The cost of developing a successful drug now varies between £3-5 million. For every drug that reaches the public, several will have failed at the stage of clinical trials and many hundreds will have fallen by the wayside at an earlier stage.

Not too many tears need be shed in response to these facts. Drug testing by the industry used to be inadequate in both quantity and quality. In the immediate aftermath of thalidomide,better testing was either adopted by or forced upon the industry. But instances of inadequate testing continued to come to light thereafter and even if those instances were the whole, rather than the tip, of an iceberg, they provided some justification for the increasing stringency with which the whole industry became regulated. There has also been justification for the mandatory introduction of new and more sensitive methods of testing whatever the costs to industry.

Without a doubt the new drugs which now reach the public are safer than they used to be. The fact that there are fewer of them not only reflects the earlier detection and removal of hazardous drugs but also the growing and justifiable bias by regulators against "me-too" drugs, which ape the successful product of a rival, and in favour of truely innovative products.

On the other hand, regulations have had an irresistible tendency to breed further regulations because, faced with the possibility that lives are at stake, any regulatory body will understandably play safe. If a better, or even just a supplementary, test can be added to those already in force, it is safest to insist that it be carried out. Would it not be better to ask for the drug to be tested in an additional species? Why not replace a three month drug dosing period by one of a life time; and then why not insist on testing for any effects on the generation—or two?

By playing safe, the regulators are protecting themselves in the event of any unforseen tragedy as well as protecting the public. It is, however, possible to be over-protective towards the public. Regulations desgined to keep out the bad can also keep out, or at least hold up, the introduction of the good. That is what the pharmaceutical industry claims has happened to an unreasonable extent.

Much of their venom is directed against the bureaucrats. Why, they ask, does it take months for the UK Committee on Safety of Medicines to decide whether or not to grant permission for a clinical trial and up to a year for approval of a marketing licence after the clinical trial has been completed? (To which a partial answer is that it takes a long time to sift through the amount of evidence that is currently demanded and even longer when the evidence is inadequate or badly presented). More fundamentally, the industry and much of the medical profession, argue that, as a way of testing for hazards, animal testing and even clinical trials have limitations which are not adequately recognised. As a result too much time and effort, not to mention money, is devoted to trying to avoid what is unavoidable at that stage.

Animal tests will never provide a complete guide to human reactions to a drug and a clinical trial can never be on scale that is sufficient to detect side reactions of low incidence. It is argued that the path of a new drug both up to and through the clinical trial stage should be made easier. In return more attention should be paid than at present to the post-marketing stage. In other words the public should opt for taking more risks because of the greater benefits that such a decision would bring, on balance.

The general line of argument in favour of relaxing regulations is persuasive, but should only be met with a cautious practical response. There may be a case for cutting back on animal testing but if so, it is only on quantity in a limited number of respects and not at all on quality. There probably is a case for allowing and encouraging regulatory bodies to give a rapid, provisional go-ahead for companies to mount a clinical trial or even to market a new drug on the basis of a summary of the evidence before it is examined in full. But that procedure should be reserved for really innovative drugs and should not remove either the obligation for the company to provide the full evidence or for the regulators to examine it and, if necessary, to withdraw their provisional permission.

Finally, better ways will have to be devised for the monitoring of drugs once they are in full scale use. Although this is widely admitted in principle, there is little doubt that it will be resisted in practice, not least because it is likely to involve a cumbersome bureaucratic mechanism. However, had an adequate monitoring system already been in operation, we would surely have been spared, in one way or another, the need for the dramatic and emotional court room drama that is currently bringing anguish to the many women who took *Bendectin* early in their present pregnancy. □

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United States

Universities push for creation of advanced energy research centres

US universities are pressing the Carter administration to help set up a number of university-based advanced energy research centres, concentrating on areas of interdisciplinary research that currently fall between the mission-oriented R&D programmes of the Department of Energy and its basic research commitments.

In his budget request to Congress for the fiscal year 1981, the President has suggested that $3 million be allocated to the department to support the initiation of such centres, an idea formally suggested only last October by Dr David Saxon, president of the University of California.

The universities hope that the administration can be persuaded to contribute more than $150 million over the next three years both to establishing 15 such centres, and to provide a national support programme of grants, traineeships and fellowships.

Administration officials are approaching the scheme cautiously, however, pointing out that the value of such centres should be judged on their scientific merits — and sceptical of any demands to institutionalise university research efforts.

The decision to provide the initial funding for a number of centres, albeit at a much lower level than the $15 million Dr Saxon had suggested, reflects a growing desire within the Department of Energy to emphasise the potential role of universities in carrying out what is referred to as "goal-oriented basic research".

In the 1981 budget request for the DoE Office of Energy Research, for example, funding for university-based energy research is scheduled to increase by 22%, a real growth of about 12%; in particular, "university research support", which includes the new interdisciplinary centres, would more than double, from $7.1 million to $14.3 million.

The specific proposals made by Dr Saxon were backed up by a report which he commissioned last August from the Association of American Universities. Prepared by a committee under Dr Jack Hollander, associate director of the University of California's Lawrence Berkeley Laboratory, the report was on Dr Saxon's desk within five weeks, and passed to Washington shortly after.

The main foci of the report are claimed gaps in the department's research strategy — and the way that universities might help to fill them. In particular, the report claims that the heavy emphasis within major R&D programmes on short-term technology development "inhibits the pursuit of goal-oriented basic research and the interaction of university researchers with the department".

In general it says that "much goal-oriented basic research tends to be considered too basic by the DoE mission-oriented programmes, and too applied by the DoE/OER basic science programmes". Examples of such research gaps, it suggests, can be found in fields such as synthetic fuels and nuclear waste disposal.

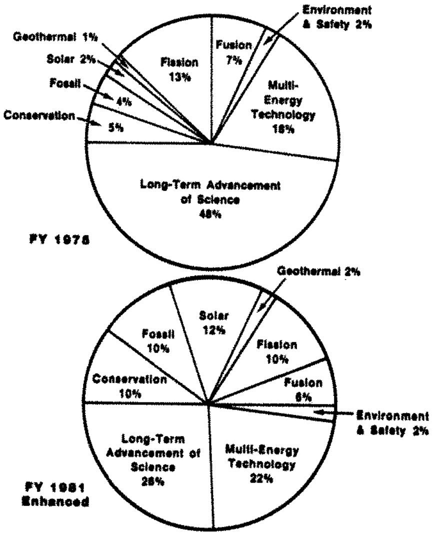
In transmitting the report to Dr Frank Press, director of the Office of Energy Research, and Dr John Deutch, undersecretary for energy, Dr Saxon spoke of the "urgent need for very substantial improvement and expansion" in the department's research strategy.

Building on the AAU report's conclusions, Dr Saxon suggested that the department agree to intiate support for five centres a year over the next three years, each centre receiving $2 million for "core support" and $1 million for scientific equipment.

Centres would be selected on the basis of a national competition. Each would build a "coordinated, coherent research programme around a central theme or subject"; styles would vary from new interdisciplinary laboratories in technical fields (such as the Materials Research Laboratories established by the Department of Defense) to interdisciplinary graduate study and research groups, such as the Energy Research Laboratory at the Massachusetts Institute of Technology.

Others might develop strong programmes applicable to a variety of energy technologies — such as research on high temperature chemistry applicable to nuclear, solar, fossil and geothermal technology — or involve research on the biomedical and social sciences related to energy supply and demand.

Shift in emphasis in Office of Energy Research support for basic energy sciences from 1975–81



The proposal was speedily processed by the DoE, and submitted in late December to the Office of Management and Budget as a last minute addition to the 1981 budget request. OMB agreed to provide $3 million for the scheme — expected to be enough to get four or five centres started — but insisted that further money would only be available if proposals are of a sufficiently high standard.

Dr Edward Frieman, director of the Office of Energy Research, told the Energy Research Advisory Board last week that proposals would have to demonstrate some special qualities, such as a strong interdisciplinary basis or close links with private industry, to qualify for funding.

"We do not want to see just a repackaging of our support for basic energy sciences", Dr Frieman said. "If the quality of the proposals is not high enough, I will not fund the programme".

Several major hurdles face the energy centres scheme, in addition to the scepticism of budget officials. One is the inevitable political infighting over where the first centres should be situated, given the traditional desire of Congressmen for 'equitable distribution' of federal dollars between geographical regions.

A second is the response of the national multipurpose laboratories supported by the DoE to a proposal that would seem to shift yet more research funding away from the laboratories into the university system. The department's budget proposal for 1981 is that the universities' share of OER funding should increase from 22% to 24%, with a corresponding reduction in the proportion of support from the national laboratories (although the latter have been provided with $60.3 million to improve basic facilities and their net funding will still increase).

Energy department officials, however, have currently taken as a key policy target the need to establish an adequate "technology base" for all its various energy-supply activities; and argue that, taking a leaf from the Defense Department's book and echoing the AAU, this can partly be achieved by emphasising goal-oriented basic enrolments research.

Universities, facing declining student enrolments and contracting research possibilities elsewhere, are keen to fit the bill. In the words of Dr Saxon: "A new programmatic departure . . . will signal the deliberate mobilisation of scientific talent in universities for the assault on energy problems of all sorts."

David Dickson

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Fast breeder reactors delayed until after 2000

A declining enthusiasm for nuclear power in the US has led the Carter administration to stretch out still further research and development efforts leading to the deployment of fast breeder nuclear reactors. These are not now planned to come into commercial use until well beyond 2000.

In his budget request to Congress two weeks ago for the fiscal year 1981, President Carter reaffirmed his previous determination to halt construction of the liquid metal fast breeder demonstration project at Clinch River in Tennessee, a move so far resisted by Congress.

At the same time, however, the President has also recommended other broad cuts in the breeder development programme not proposed in previous years. This is in line with a decision taken late last year to stretch out the LMFBR programme and defer building breeder reactors, previously expected to come into use in the 1990s.

Administration officials are thought to believe that, given the current uncertainties surrounding the future of nuclear power, following the Three Mile Island accident, as well as adequate domestic supplies of uranium, it would probably be impossible at present for a new reactor type to be produced and licensed in the US. The Department of Energy had asked the White House to keep breeder funding at the 1980 level, apart from Clinch River.

Recommended cuts in the breeder programme include reducing support for LMFBR technology from $140 million to $80 million in 1981, and cutting out completely further development of the gas-cooled breeder reactor, currently being supported as a back-up programme.

Funding for research and development in fuel processing technologies for breeders is proposed to be reduced from $49.5 to $12 million, and plans for a conceptual design study for a larger scale plant than the Clinch River project will be terminated.

Overall funding for the breeder programme will, if Congress agrees, be cut from $761 million to $384 million. What actually happens, however, will depend on the outcome of the conflict over the Clinch River funding; until this is resolved, the administration is facing severe difficulties in producing a balanced programme for nuclear fission research.

Domestic cutbacks in the breeder programme have heightened the enthusiasm of energy officials for cooperation with other countries. In particular an exchange of agreement was signed with France at the beginning of December, through which it is hoped to work with French scientists and engineers. □

Canada

Election candidates asked for science funding commitments

Canadian scientists are mounting an active campaign to inform candidates in next week's general election of the need to increase support for university research. **David Dickson** reports

SOON after Canada's nine-month-old Conservative government resigned last month, scientists at the University of Toronto placed a letter on campus noticeboards asking for volunteers to investigate the attitudes of local candidates in next week's general election towards research funding.

Each volunteer is being equipped with a background kit on federal R&D policies prepared by groups such as the Canadian Association of University Teachers and the Canadian Federation of Biological Societies, and then assigned to candidates in one of Toronto's 23 ridings (constituencies).

The idea is to discover whether in general terms a candidate is prepared to support increased funding for research and development — or to provide information to those who have not considered the issue before — and also to report back on any commitments that a candidate may make.

These are checked back with the candidate's party, and noted for future use. "We are using this as a device to wheedle commitments, and if we get the commitments we are going to nail them," says Dr Ron Clarke, director of the University of Toronto's Office of Research Administration.

The scientists' campaign is being organised jointly by the CAUT, the CFBS, and other professional societies such as the Canadian Association of Physicists. With lobbying planned in more than 50 election districts, it is the latest tactic in an aggressive grass-roots strategy waged over the past few years to focus political attention on the relative lack of funding for university research.

Argument has been focused on the fact that government spending on basic research fell from 0.1% to 0.07% of the gross national product between 1969 and 1979, less than half that of most industrialised nations.

Responding to such criticisms, the Liberal government made a commitment to increase overall R&D spending to 1.5% GNP by 1983 shortly before losing power last year; and in November, Mr Howard Grafftey, Minister of State for Science and Technology, announced that the Conservative government proposed to increase the funding of the Natural Sciences and Engineering Research Council by 32%, to a total of $159.8 million, in the fiscal year 1980-81.

This increase was contained in the total budget figures over which the Conservative government was defeated in Parliament in December. But last week, with the election campaign in full swing, Mr Grafftey announced that NSERC's budget would be increased still further, to a total of $162.6 million.

At the same time, Mr Grafftey announced that the budget of the Social Sciences and Humanities Research Council would be increased by 16.2%, and that of the Medical Research Council by 17.4%bringing the total increase for the three research councils to 26.4%.

Budgetary levels for the councils approved by the Cabinet are for the first year of five-year plans which each has submitted. The increased funding has been welcomed by the scientific community, which feels that, whichever party wins next week's election, it will be difficult to go back to the lower increases previously projected. But there is concern over whether the increased level of funding will be maintained into future years.

The Party has publicly committed itself to an eventual target of spending 2.5% of the gross national product on R&D, although without being specific about timing. The small but growing New Democratic Party — which could hold the balance of power in a close ballot — has adopted the ambitious target of 4% by 1990 and 2.5% by 1983.

Many scientists doubt whether these are realistic targets, given the rapid sustained growth in funding that they would require. But there is determination to keep pressure on the new government to confer stability to the Canadian research effort.

One element of concern is that the new government should retain a minister with full-time responsibilities for science and technology. Another is that the provinces should play their part in the national effort to stimulate R&D; some have recently been criticised for diverting federal funds allocated — although not mandated — for the support of further and higher education.

In Toronto, the scientists claim their grass-roots lobbying towards such objectives is beginning to pay-off. Already one Liberal ex-Cabinet member has said his party, if elected, will appoint a full-time minister for science and technology. But what actually happens after election day remains to be seen. □

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Poland

Science planning praised — but improvements still needed

SCIENCE planners and civil servants in the science-related ministries should improve their scientific qualifications, according to the "guidelines" for the Eighth Congress of the Polish United Workers' Party this week.

The "guidelines", which after due discussion will form the basis for the 1981-85 Five Year Plan, initially paint a glowing picture of successes scored by Poland's special approach to science planning, with its hierarchical structure of research tasks, from "Government" and "Key" down to "Departmental" problems. These achievements range from laser thermonuclear microsynthesis to mining tectonics, the mapping of Poland's flora and fauna, and the use of "Arctic Marine organisms" (ie krill) as a source of protein.

But it does seem that the "problem solving" structure of Polish research has not yet overcome the difficulty endemic throughout the Comecon bloc — that of implementing R & D results.

"It is absolutely necessary", say the guidelines "to introduce a system enabling universal incorporation of Polish inventions in national research and development endeavours. Contacts and cooperation among scientists, engineers and workers must be strengthened as an important vehicle of propagating technical progress and enriching the scope of innovations in industrial plants."

When it comes to the details of research planning, however, the "guidelines" show a certain ambivalence. On the one hand "the realisation of long-range research plans requires their closer connection to development programmes of the corresponding fields of the national economy". On the other, "more flexible methods of work must be developed in scientific as well as research and development units", and "the manner in which scientific research establishments are organised, their employment structure and planning methods, must be adjusted to changing needs."

To enable them to strike the proper balance between these demands, the staff of the relevant ministries are urged to continue their higher education "in cooperation with the institutes of the Polish Academy of Sciences and university-related schools".

The "guidelines" propose a number of "tasks" for the special attention of the science and technology planners. These come as no surprise, either within a world context (limitation of petroleum consumption, development of "unconventional" energy sources, and environmental protection) or within the special conditions of Poland (water management, development of high-protein and oleaginous plants, the development of specialised exports to the highly developed countries, and the export of Polish technical know-how).

But perhaps the most interesting "task" is "the production of pharmaceuticals . . . through the more extensive use of domestic syntheses and achievements in the field of genetics", a field in which, according to a recent article in *Polityka*, the commercial breakthrough point may be expected in the near future.

Vera Rich

United Kingdom

Study finds malnutrition in 7% of elderly

NUTRITIONAL problems among Britain's elderly population are highlighted in a report* published recently. "Nutrition and Health in Old Age", is the result of a survey of the nutritional status of 365 old people in 1972-73. The incidence of malnutrition was 26 (7%). It was twice as common in subjects over 80 years old.

Malnutrition was associated with both medical and social risk factors. Medical conditions included chronic bronchitis, dementia, depression, difficulty in swallowing, and poor dentition. The social risk factors included being housebound, living alone without regular cooked meals, bereavement, and being dependent on welfare benefits — though the report does say that "lack of money did not appear to be the sole cause of undernutrition.

Among the 26 were three with scurvy, two with osteomalacia, and 13 with nutritional anaemia associated with haematological evidence of iron, folate, or vitamin B_{12} deficiency. The remaining cases showed evidence of multiple nutrient deficiency. Diagnosis was drawn from clinical, dietary, biochemical and haematological studies; single clinical signs and isolated biochemical findings were considered to be unreliable.

The subjects were not a random population and the conclusions cannot be extrapolated to the population as a whole.

Alastair Hay

**Nutrition and Health in Old Age. Report on Health and Social Subjects, Volume 16. DHSS. Available from HMSO at £5.25.*

The Netherlands

Recombinant DNA research thrives

RECOMBINANT DNA research in The Netherlands is expanding at a rapid rate. At the end of 1976 only seven projects at universities had been registered by the commission of experts, established by the Royal Academy of Sciences in 1975 to look at genetic manipulation. By the end of 1979, there were 78 such projects, of which about 10 are with industrial companies, such as Unilever Research. All this research, according to the commission's latest annual report just published, will take place in category I and II laboratories.

The work of the Academy's commission has now been taken over by a government commission whose members include representatives of trade unions, the health authorities and industry. The Academy commission relaxed the safety guidelines in 1978. These came into effect a year ago and were extended to include yeast as a host organism. The new commission has already announced that it will consider a further relaxation of the regulations.

The chairman of the first commission — in a farewell speech — emphasised the need to build a category III facility which researchers say is needed in the country. The lack of such a laboratory has resulted in a falling behind in important research on, for example, cancer-inducing viruses and genetic aberrations, fields in which until recently The Netherlands had made progress. Even with a further relaxation of the regulations, Dutch scientists say that a category III laboratory is essential for research using DNA of warm-blooded animals and, in particular, man.

The previously strict rules in the country did not result in the departure of Dutch scientists to work abroad. Only seven researchers left temporarily to work in a category II or III facility, and five of them also asked foreign laboratories to work for them. But, in its last report, the commission says that it would be wrong to conclude that molecular biological research in The Netherlands had not suffered from the strict guidelines in force until recently.

In The Netherlands, especially in industrial circles, there is still a lot of dissatisfaction about the conditions in which molecular biologists must work. The public debate which reached a peak in June 1978 has now died down, and among scientists there is very little opposition to the relaxing of the guidelines. A long-awaited special committee to study the ethics and social aspects of work with genetic material has still not been set up by the Dutch government, but this is expected soon.

Casper Schuuring

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Dioxin

Chemical company study shows no dioxin hazard

A REPORT to be published this month claims that workers exposed to significant quantities of the animal carcinogen 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) over thirty years ago have a normal incidence of cancer, and a reduced incidence of cardiovascular disease.

The report, published in the American *Journal of Occupational Medicine,* is an epidemiological survey of workers exposed to dioxin at the Monsanto Chemical Company's site at Nypro, West Virginia. 228 men are known to have been exposed to the chemical: 117 as a result of an accident in the 2,4,5-thrichlorophenol plant on 8 March 1949, and 111 in the routine manufacture of the herbicide 2,4,5-T. It documents the mortality indices for 121 of the 228 workers who were traced.

Acknowledging that these results are only part of the story, the report's authors — Judith Zack, an epidemiologist employed by Monsanto and Raymond Suskind, Director of the Institute of Environmental Health at the University of Cincinnati Medical Centre — say that details about the other workers will be published in due course.

Publication of this information by Monsanto is a welcome departure from the company's previous position of treating all information about these workers as confidential. Little was known about the Monsanto accident until July 1976 when a trichlorophenol reactor at the Italian town of Sèveso overheated, discharging dioxin over the town and its environs. In the aftermath of Sèveso, details began to emerge of at least 16 similar accidents in various parts of the world. Accounts of these accidents and their consequences were requested by Italian officials and scientists anxious to ascertain the consequences of the dioxin exposure for the people of Sèveso. Sadly, few companies have been forthcoming; this latest initiative on the part of Monsanto may have the effect of breaking the log jam.

A Monsanto spokesman described this latest report as "reassuring". But it is unlikely to allay all fears about the toxic properties of dioxin. For the results of the Monsanto survey differ from those following surveys of workers exposed to dioxin in West Germany and Holland. Workers known to have been exposed at Badische Anilin und Soda Fabriks' Ludwigshaven site in 1953 are reported to have suffered from a high incidence of gastrointestinal cancer, whilst others contaminated by the chemical at the Amsterdam site of Philips Duphar in 1963 are recorded as having a higher incidence of heart attacks (*Nature* 19 January 1978, p202).

Zack and Suskind note that their results do not agree with those obtained for German and Dutch workers. They also acknowledge that their survey does not confirm the higher than normal incidence of bronchiogenic carcinoma in workers exposed to dioxin in an accident in Czechoslovakia in 1964.

More recent evidence about the potential carcinogenic properties of dioxin are reported in a survey of Swedish forestry workers published in the *British Journal of Cancer* **39**, 711; 1979. According to its authors, Dr Lennart Hardell and Anita Sandström of the Center of Oncology at the University of Umea, exposure to dioxin contaminated 2,4,5-T or chlorinated phenols could be responsible for the six-fold higher incidence of soft tissue sarcomas seen in these men.

With so much conflicting evidence about the long-term effects of exposure to dioxin, the results of the Monsanto survey — albeit the largest so far — are unlikely to still fears about the risk posed by the chemical. The US Environmental Protection Agency has instituted a temporary ban on 2,4,5-T. Whether or not that ban should be made permanent will be decided after an Agency hearing on the herbicide due to begin on 11 March. The EPA will have to make sense of this conflicting data.

Alastair Hay

Mediterranean

No cash to clean up pollution

MEDITERRANEAN states have come up with only one third of the money needed to maintain the United Nations Environment Programme's Mediterranean pollution programme, UNEP's deputy director Peter Thacher announced in Barcelona on Monday.

Speaking at a meeting of the 17 signatories of the 1975 'Barcelona convention' on pollution in the Mediterranean, Mr Thacher warned "we have today only seven weeks left before we must stop" a number of the programme's activities. Jobs in the programme's 12-strong secretariat are also threatened: "a number of critical posts are filled on contracts which will expire in March unless new cash contributions are received", said Thacher.

A year ago, a meeting of the signatories in Geneva agreed to establish a trust fund of $6,480,000 to guarantee the programme for the two years 1979-80. The 17 states, plus the European Commission, were to contribute half, UNEP one quarter, and other UN agencies one quarter in goods and services. A year later, eight countries and the EEC had contributed nothing.

Only five countries have contributed in full, and these were among the smallest and poorest: Algeria, Tunisia, Cyprus, Malta, and Greece, providing $130,000 among them.

Among the major countries France has been the most forthcoming, with $990,000 of its agreed $1,564,000. Italy and Spain, however, which committed themselves at Geneva to spend $744,000 and $412,000, have so far contributed nothing, said Thacher. In total the countries have contributed $1,150,000 against an expected $3,200,000.

The next treaty to be signed is the treaty on land-based sources of pollution, the crown jewel of the Mediterranean programme. It was expected that states would sign the protocol in Athens this May and ratify it within two years, but, said Thacher, there were not even enough funds in the kitty to convene the conference.

Moreover land-based sources, particularly of untreated sewage, are the major cause of pollution in the Mediterranean, and would cost — according to rough estimates — some $15 billion to clean up. UNEP officials have been assuming that this money would be spent over some 10-20 years, representing a $1.5 billion to $750 million-a-year spend, 500-1000 times the present — and unachieved — target.

The 'Blue Plan', a proposed high-level economic and sociological study of the likely development of the Mediterranean region to the year 2000, is also threatened, though not by lack of cash. States had been asked last February to nominate two candidates each for six posts to implement the Blue Plan, but two months past the deadline only seven nominations have been received from seven countries. And, said Thacher, "very few of these candidates have sufficient academic qualifications or experience". He concluded that governments were "not serious" about the plan.

- In an extension of the UNEP Regional Seas Programme (of which the Mediterranean programme is a part) to the Caribbean, experts from 23 mainland and island states of the region have agreed to recommend to their governments an action plan on pollution and development. "In its economic and social aspects, the Caribbean Action Plan goes well beyond the Mediterranean Action Plan" says UNEP. The first governmental meeting on the plan will be held in September.

Robert Walgate

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NEWS IN BRIEF

US city bans nuclear waste shipments

THE city of Missoula, Montana (population 58,000) banned last week all shipments of nuclear materials through the city limits. Citing two accidents involving trucks carrying nuclear wastes that occurred this winter on Interstate 90 — which runs through the city's northern boundary — as evidence of the hazard involved, the City Council passed the ban by a vote of 7:1. The measure is expected to interfere with the shipment of wastes to the Hanford nuclear plant in eastern Washington. Included in recent shipments to Hanford are wastes from the accident at Three Mile Island in Pennsylvania.

Séveso director killed by political group

THREE men and a woman, ambushed Paolo Paoletti, aged 39, a director of the ICMESA chemical plant in Séveso outside his Monza home last week. The Italian urban guerrilla group, Front Line, claimed responsibility for the assassination. Paoletti was one of several senior ICMESA officials arrested and charged with operating a dangerous factory after four pounds of dioxin were released when a chemical reactor exploded in the factory in 1976. More than 600 children developed acute cases of chloracne as a result of exposure to dioxin. The legal case is still pending with the parent company Hoffman-La Roche of Switzerland expected to admit responsibility for damages caused, but not negligence.

UK radiation study overestimates safety

A BIENNIAL radiation survey conducted by the UK Health and Safety Executive has seriously underestimated the exposure to the workforce at 11 UK nuclear power stations by its exclusion of part-time workers from the study. Although claiming that none of the 7500 nuclear plant workers in either 1977 or 1978 received more than the international limit of 5 rems, the report did not cover an estimated 1500 temporary employees, many of whom were hired to do the hazardous work of pipe delagging in areas heavily exposed to radiation. It is not uncommon in pipe delagging operations for workers to receive their allowed quarterly dose of 2.5 rem in less than two weeks. These workers are then replaced by other temporary employees. An HSE official confirmed that the study did not cover "employees not directly employed by the Central Electricity Generating Board" and said that no figures were available on the numbers hired to do delagging or on the radiation doses they had received.

Health and Safety: Nuclear Establishments 1977 — 1978 HMSO £1.25.

More UK nuclear cracks

BOTH gas cooled reactors at Britain's Bradwell nuclear plant in Essex have been shut down to investigate cracks in the welds of the expansion bellows of the primary cooling circuit similar to the ones found in the Dungeness reactors two weeks ago (*Nature*, 17 January, page 238). The first Bradwell reactor had been shut down for routine maintenance when the cracks were discovered. As a safety measure the second reactor was shut down and similar cracks were discovered there as well. The reactors could be off line for a year or more.

UK centre for analysis of technical change

A NEW research centre for analysing technical change with the goal of "increasing national wealth" is to be set up by the Leverhulme Trust in conjunction with the Science Research Council and the Social Science Research Council. Leverhulme will contribute £1.5m towards the centre and the two research councils will each contribute £½ million over a period of the first five years followed by £250,000 a year for the second five years. Sir Michael Swann, FRS, will chair the organising committee and the centre's governing board. The types of issues to be considered include energy and material resources, manufacturing technology and unemployment. The majority of the proposed staff of 20 professionals and 10 supporting staff will be on secondment or will have joint appointments with universities, companies or government establishments.

Vietnam veteran records Agent Orange testimony

CHARLES HARTZ, a US Vietnam war veteran, who was exposed to the dioxin containing defoliant Agent Orange, initiated a massive law suit against the manufacturers of the chemical in Philadelphia last week. Hartz, who suffers from terminal brain cancer, videotaped his testimony accusing the manufacturers of negligence in not testing the chemical for its effect on humans. The pre-trial session had been organised by Hartz' attorney out of fears that Hartz will not live to testify when the trial begins later this year. In spite of objections from the manufacturers, District Judge George Pratt ruled that video tape was the best way of preserving Hartz' testimony. The law suit against Dow, Monsanto, Hercules, Diamond Shamrock and Thompson Heyward is presently being organised by 3000 exposed veterans but is still open to others.

20,000 demonstrate against Brittany reactor

TWENTY thousand demonstrators carrying French and Breton flags braved wind and rain squalls to march from the town hall in Plogoff to the site of a proposed nuclear reactor on Cap Sizan on the wild Brittany coast. Called to form part of a continuous protest to six weeks of hearings lasting until 14 March, the demonstrators plastered the town with slogans including a reminder to the government of its promise of April 1974 that "nuclear power will not be imposed on a population that refuses it".

Sussex physicist appeals to Queen over job

DR ROBERT Golub, a 42-year-old American physicist working at the University of Sussex, has appealed to Her Majesty the Queen over the termination of his research fellowhip. The Queen is 'visitor' to the university, a role following from its charitable status; in principle she acts as an ombudsman, caring for "breaches of the rules of natural justice" (in the words of one legal expert) but not for matters of academic merit.

Golub's research fellowship, which is payed for by the Science Research Council, has been renewed regularly since 1968 for his work on ultra-cold neutrons. A new neutron source he devised, using superfluid helium, is to be installed at the Institut Laue Langevin, Grenoble, for 700,000FF (£80,000), but without his assistance. Golub believes that an industrial tribunal would dismiss his case because of a clause in his contract which waives his redundancy rights.

The Privy Council has written to Golub telling him that it could intervene only if the university was guilty of "incorrect procedures or improper motives".

"I don't think you've much chance! Look who's in front of us in the queue!"

0028-0836/80/07614-01$01.00

Low expectations for Swedish PhDs

In Sweden, as in most European countries, young scientists are facing problems finding work. **Wendy Barnaby** reports on the falling market in research positions for newly-qualified PhDs

THE expansion in university teaching in the 1960s has not been followed by the creation of research posts for today's postgraduates, and many young PhDs in the natural sciences are finding it very hard to do research.

The expectations of young Swedish scientists have certainly fallen, yet they are hardly ever — strictly speaking — unemployed. According to Dr Bengterik Ronne, at the central administration of universities and tertiary education institutions (UHÄ), PhDs can still get jobs but they have to start at a lower level than they would have done a few years ago. This is borne out by figures published by the National Central Bureau of Statistics, showing unemployment amongst all licentiates (a qualification, now abolished, a little lower than a PhD) and PhDs, three years after graduation.

The latest available statistics cover the period 1976–1978, and continue a reasonably unchanging pattern in which most PhDs (as high as 90% to 98%, for this period) say they are employed wholly or partly within the field for which they were educated, with relatively qualified tasks. Only eight people over the whole period, out of a total of 1,008, said they were unemployed, and there is no way of knowing from the statistics whether they were licentiates or PhDs, or whether they had read social or natural sciences. But the statistics hide a lot of discontent.

Not many PhDs go into industry. "In the 1960s, Swedish industries were very optimistic about the numbers of PhDs they would need", says Professor Ingvar Lindqvist, Secretary of the Natural Sciences Research Council (NFR); "but when economic conditions changed for the worse, they realised they had overestimated their needs, and both industry and government lost interest in PhDs." According to Bengt Gustafsson at the central organisation of the academics' trade union (SACO/SR), there are only 700 PhDs working in Swedish industry, and most of them are chemists doing research in pharmaceutical companies.

Most natural sciences PhDs, then, end up in universities. But conditions there for them are far from good. The 185 who received their doctorates in 1976/77 had less than a one in ten chance of getting a research position (*forskarassistent*), which can be held for two three-year periods. A small number of PhDs are given grants by the NFR and hope to be supported by NFR funds for their entire careers.

Other options are to apply for grants from other foundations or become attached to research projects that are already funded. But all grants are very insecure, as Professor Lindqvist complains. "There is a general political philosophy in this country of security in jobs", he points out, "but it hasn't worked well at the universities. People get grants for one year at a time."

Under the security of employment laws in Sweden, a university is obliged to offer a research scientist another job at the same salary — either in his own department or in another one — if his grant dries up. But trying to find niches for displaced people can be a difficult problem, as one of the SACO/SR representatives at the Royal Institute of Technology (KTH), Dr Bertil Wilner, has found. "It's easier to find alternative positions for technicians and administrative staff", he says, "but for specialised research scientists it's a very hard problem to solve." He estimates that about 5% of KTH's research scientists find themselves in this situation every year. The security of employment laws provide legal assurance of an uninterrupted income but not of uninterrupted research in the same field: there is simply not enough research money to assure that.

Money is a problem at all levels of research. At the end of 1978, the NFR asked an international group of physicists to evaluate Swedish experimental nuclear and particle physics. "The first and overwhelming impression", reported the group, "was one of underfunding . . . More precisely, the present materials budgets for research per physicist are too low to guarantee efficient work. They are much lower than current standards internationally, most likely by a factor of two or three."

The group also commented on what it considered the "old fashioned organisation of research at the universities. Younger researchers with international reputations seemed unable to command permanent research positions. The near absence of intermediate tenure positions is disruptive and unhealthy for all long and medium term programmes. The present situation leads to an obsession with the necessity for personal research programmes with too many subcritical groups."

All this insecurity is having a distinct effect on PhD students, many of whom are increasingly reluctant to finish their doctorates. The PhD student is marginally more secure than the PhD graduate, even if, as UHÄ maintains, some students are forced to earn a living by taking on research not particularly suitable for their doctorates — another contributing factor to the decline in PhD examinations.

Since 1971, the numbers of natural sciences students passing their PhDs has dropped every year; and in 1976/77 it was less than in the mid-1960s even though recruitment to research education has remained fairly constant.

So far, the effects of these problems on research as a whole are not easy to point to. The international evaluation of physics made by the NFR, for example, did not mention any area in which Swedish research in physics is falling behind that in other countries, even though the evaluators often expressed their surprise about personnel and organisational set-ups; and Professor Lindqvist cannot point to any areas in which Swedish scientists are lagging.

But there are problems in sectoral research — research supported by organisations within their own fields of responsibility. According to Professor Erik Arrhenius at the Department of Cellular Toxicology, University of Stockholm, it is very difficult to get PhD students involved in research on the environment. The projects supported by the National Environment Protection Board (NEPB) run for one year at a time, and good scientists want more security than a one-year project can give.

The National Board for Technical Development (STU) has had similar problems with one-year projects. According to Dr Lennart Lindeborg, these problems have triggered the setting up of a new arrangement under which STU will guarantee the researchers' involvement in future projects for five years. The new projects will be in electronics, informatics, speech communication, food production processes, gene technology, the chemistry of wood, enzyme technology, the physics and chemistry of surfaces, fibrous materials and future industrial production systems.

Apart from the plight of present researchers, many are worried about the future. There is a fear that recruitment of graduates for PhD studies will decline. SACO/SR points to the slide in students

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completing three years of natural science subjects at universities from 1,390 in 1970/71 to 564 in 1976/77. Bengt Gustafsson maintains that this drop has resulted from the decline in university intake in 1972/73. As the numbers of new students fell off, so did the money for teaching staff; so the universities tried to make themselves more interesting by setting up short courses such as popular astronomy and amateur geology.

These courses have proved almost too successful: students now take them in preference to long courses that can lead to research. Professor Lindqvist thinks there will be a shortfall of PhD recruits in 15 to 20 years. He points to the increasing unpopularity of the natural sciences in secondary schools, saying that it is much easier to earn maximum marks (and it is marks which count for university entrance) in non-science subjects.

There have been piles of papers written about the reorganisation of the universities. Two more commissions will be reporting soon — one on the reorganisation of teaching structures and the other on the education of research students. The first commission is expected to recommend widespread changes to create more tenured research positions and spread the teaching burden more evenly amongst university jobs.

But the crux of the matter is money. Last year, Parliament allocated the UHÄ Sk205 million (about $46 million) to provide five universities with basic facilities with which to conduct natural sciences research: research education, fundamental research, professorships and appointments for those who had passed their PhDs, secretaries, libraries, instruments and buildings. Engineering research received Sk222 million (about $50 million), to be spread over six technical universities, for the same sorts of things.

As salaries, equipment and research funds all come out of the same budget, research gets less money as the universities have to go on paying the salaries of scientists whose projects have ended. According to Professor Lindqvist, the University of Lund has to pay more than SK2 million (about $450,000) a year supporting former docents.

All the relevant authorities are demanding more money, and all the political parties said before last September's election that more money should be given. The Swedish Parliament has in fact agreed that about 1,000 jobs in the public sector — in archives, museums, libraries, positions in planning, commissions of enquiry and some teaching posts at secondary schools — should be given in the first instance to PhDs.

The union also wants the state to give increased support to the development of technology-intensive industries such as telecommunications, energy and defence, to provide secure working places for scientists as well as competitive exports. □

How the popularisation of science narrows the polarisation of the people

In the last of three articles on science and technology in China, **Tong B Tang**, research fellow at Darwin College, Cambridge, UK, reports on scientific education and popularisation

Workers from three provinces of North East China watching a lathe demonstration at a gathering in Shenyang

EDUCATION has always been a central issue in Red China. By comparison, Marxist socialism in the West was also initially strongly associated with an educational movement; it stimulated developments in the philosophy of education, and was instrumental in spreading the concept of education for the working classes. This last influence was never necessary in China: in the past, non-hereditary mandarins were selected by imperial examinations which theoretically anyone could take. Traditionally the populace has been conditioned to appreciate the advantages of education. This deep-rooted exaltation of learning however, makes it all the more important to watch out for intellectual (as well as bureaucratic) elitism.

Two years ago China introduced a nation-wide examination for university entrance to replace nomination at one's place of work. Had everyone equal access to primary education, there would be equal opportunities for all. In practice however, students who can or want to take advantage of further education still tend to come from professional families. Another uneven situation is the formal existence of "key schools", (and universities) which admit "bright" pupils (from the age of five or six onwards) and whose facilities are given priority.

Going straight from high school to university is no longer rare but the long-standing policy of mobilising school leavers to work or settle in the countryside still persists, as affirmed by Chairman Hua in August last year. There are still many technical problems but this policy, besides playing other roles, spreads learning to the rural masses.

Another effective way in which the Chinese try to redress any imbalances in educational equality is their system of marking the university entrance exam. The pass mark is regionally adjusted, and lowered for candidates who have left school and are working in the countryside; ethnic minorities are also given 10 additional marks and educational work in secluded and border areas is paid special attention. Depending on the university (higher for a "key" one), the students need 300-400 out of a possible total of 500 marks to get in. Once there, freshers who come from poorer backgrounds are given extra

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tuition.

At present formal degrees are not awarded at the end of the period of study. This will probably change soon: undergraduates sent abroad are reading for degrees. The younger of those scientists sent to participate in research are also encouraged to register for postgraduate degress; the first Chinese to receive a PhD did so last September, in West Germany. The course structure, originally modelled on the Russian system which emphasises early specialisation, is being modified. Nanjing University, for example, introduced a credit system in 1978 similar to that used in US universities, where the student is freer to choose his or her courses. Summer schools at the pre- or post-doctoral level are also being planned.

At the same time, the Chinese are putting a lot of effort into updating teaching materials: there was a conference on this topic early last year. In most subjects, there are revised editions of former textbooks in circulation, and many universities are now trying them out. (In China most schools use a unified set of texts, while for universities about 70% of the curriculum is standardised by the National Planning Committee in the Ministry of Education.) A selection of course books recommended at major overseas universities is also being looked at. Some of these books may be purchased in bulk when the students are adequately prepared in the relevant foreign languages.

During the past decade most schools and university departments became linked to communes or factories, where pupils and students went periodically to work as part of their education. This scheme has ceased, since their main duty is now assessed to be academic study; so has the "inviting in" of workers and peasants who brought in grass-roots experience and argued with the students and their teachers. However, high-technology pilot plants or small manufacturing units, which provide contacts with workers can still be found in science faculties. More crucially, the long-developed tradition of political work has not disappeared. An academic works for six days a week, one of which is devoted to political study which may include physical labour on the campus. For students in any faculty, there is a 'core curriculum' of philosophy, political economy, Party history and the history of the international communist movement. It accounts for about 200 hours out of a total 2,500 curriculum hours and carries 8 to 10% of overall marks.

以阶级斗争为纲
坚持以工人为主体的三结合
加速发展六米大型水泵

Workers in Shanghai examining a water pump design

With a population of 1,000 million, China has 10 million teachers for 210 million students. Of these, respectively, only 0.2 and 0.9 millions are in the 598 post-secondary institutions. As a medium-term measure to achieve a rapid expansion of tertiary education, an increase has been made in the number of students sent abroad. There are at present 180 postgraduates and 420 undergraduates in North America, Japan and Europe, 80% of whom are in natural sciences. By comparison, 1,300 from the West and the Third World are studying in China. An unusual measure, further, is that a few self-paying students who can afford it are allowed to go abroad; some think that this may be seen to serve the principle of "to each according to his capital".

Scientists and peasants preparing a simplified medium for pollen breeding at the Institute of Genetics of the Academia Sinica

The opposite tendency, namely, "to each according to his needs", is fostered by a mass movement to raise the scientific and cultural level of the entire nation.

The objectives of science popularisation are not confined to education. It serves to drive out the out-dated ideas in the social consciousness of the whole people, and to generate greater enthusiasm for the Four Modernisations (in agriculture, industry, national defence, and science and technology). The idea is that science popularisation will reduce social polarisation by narrowing the differences between city and countryside, and by reducing the alienation due to the division of labour.

The organisation of popularisation is largely, though not exclusively, in the hands of provincial chapters of the Scientific and Technical Association which are directly responsible to local authorities.

The activities take many forms. In children's and youth clubs, science classes are held and model or instrument building competitions are regularly sponsored. Summer and winter camps teach subjects that range from geology to ecology. Educational exhibitions are shown in the camps too, and in public parks. Public lectures are frequent, sometimes by such leading scientists as Qian Sanquiang whom some foreigners dub the "father of atomic bombs in China".

There are popular science magazines and newspapers on sale throughout the country. No fewer than 1800 popular science books have been published since the beginning of 1978. In August 1979, an Association of Science Popularisation Writers was formed.

Science fiction based on hard science only is expected to flourish soon. Posters of great scientists are constantly in print. I saw the following: Newton, Einstein, Copernicus, Marie Curie, Darwin and the inventor Edison. But disappointingly I didn't find one of an ancient Chinese scientist. Visual arts with scientific themes are also being encouraged. More than 100 new educational films were scheduled last year.

Since May last year "universities of the air" have been started in 29 local TV and radio stations. Correspondence courses are also being extended. In a few years' time there may be twice as many students in these alternative forms of education than in the regular universities and colleges. Full-time schools are also being run to give technical instruction to administrative cadres. Party secretaries and bureau chiefs from the provincial level down and leading personnel in factories, mines and oilfields attend courses lasting two weeks to four months, in farm economics and machinery, stock-breeding, fishery, forestry, enterprise management or computers. It would be amusing for the mandarins in the British Treasury to have to go to evening classes in arithmetic.

It would be unhelpful and unrealistic to imagine that despite their enthusiasm, the Chinese have no major problems ahead. As reiterated in a recent editorial in the People's Daily, the question of manpower is the foremost obstacle to the modernisation programme, and to education in particular. Many scientists have little experience of research management: here the Chinese can learn a lot from the West provided they are selective. In the transformation of education is the teachers who constitute the single most crucial sector.

If the development of Chinese science is dependent on politics, then over the next two decades, China might well fulfil its aim of developing a science of its own. It has to be careful to avoid a Scylla of Westernisation and the Charylbdis of misguided dogmatism. The Chinese believe that the human activities grouped under the name of science are intimately related to other processes in society — "if society has a technical need, that helps science forward more than ten universities"; at the same time, dialectically the reverse is also true. Science acts on the technico-economic base and is therefore partially self-supporting. □

Friends and foes of science debate in France

Jim Ritter records some observations on what was "effectively the first public debate on science and technology in France for more than a decade". It was staged last month, by *Les Amis de la Terre.*

THERE is a sense of movement about French scientific policy today, a certain loosening of the tight strictures of official silence and lay apathy. What public debate there has been about science has taken place within the cloistered university circles of philosophers and sociologists, while science policy decisions continue to be taken in equal isolation by scientific and political mandarins.

Hence the strangeness to the Anglo-Saxon eye of the programme for a debate on science and technology policy organised by *Les Amis de la Terre* and held in Paris one weekend last month. There was little discussion of concrete points of technology — nuclear power safety, pollution, and so on. There was much of more epistemological questions — the nature of quantum mechanics, the question of systems analysis. But the main stress was biological; a reaction to the "biological determinism" now being vigorously propagated in France by a group of influential far-Right intellectuals (the New Right) who claim that modern biology has "proven" the inequality of races, sexes, and classes. The organisers did well in assembling, for virtually the first time in a decade, a number of top administrators, active researchers, authors and journalists to face a concerned public.

The debate started with a panel discussion among three physicist-philosophers on the interpretation of quantum mechanics. The hall was surprisingly full and the question of whether the Copenhagen interpretation was idealist or not was entered into with great gusto. But the debate was inconclusive, the question remaining rather elusive though the level of debate was one which, in the UK, would only be found in specialised seminars.

The afternoon session on bioengineering was livelier and more familiar. The panel consisted of three directors of biological research and two young researchers. The difference of opinion ran along the same line, the mandarins seeing no reason why genetic manipulation should not be prosecuted with all possible speed while the two opponents pointed out what they saw as disquieting experimental evidence and questioned the point of research. The audience of more than 100 readily joined in. They were clearly worried while the administrators were equally clearly unused to hostile questions and overracted. The scientists quoted particular experiments to support their claim that genetic manipulation was/was not dangerous but never seemed to come to grips either with each other's claims or with the general unease among the lay members of the audience.

Sociobiology, in the next session, generated a more unanimous attitude, with two of the three biologists on the panel having written recent popular books on the subject. A clear presentation of the basics of the theory, together with an uncovering of the political attitudes underlying its "objective, scientific" attack on women, non-whites, and the poor, led to one of the liveliest debates of the weekend. The discussion was continued into Saturday evening with further analysis of the particular use of women as a target by the New Right.

Sunday morning and it was the turn of systems analysis. Here both the panel and the audience were divided on the merits of a general systems approach. Some saw it as the answer, some would replace it with catastrophe theory, some saw any such search for a totalising theory which explains all levels of the universe at once as obfuscation. The search for and critique of panaceas went on into the following physics debate. Are microprocessors the solution or the problem? Will larger and larger particle accelerators advance physics as much as more modest investments elsewhere?

The final session was on social responsibility and the hall was packed to overflowing, the debate heated. The presence of trade union representatives and members of various political and environmental groups made this discussion the most familiar to the Anglo-American ear. But when one participant pointed out that "we've heard this all before, ten years ago", one was reminded of just how damaging the long silence in French public debate has been.

In any case the science policy debate has reopened in France after a long hiatus. Any facile optimism about how easy it would be to simply confront the "expert" and "the public" has evaporated after 20 hours of long and arduous discussion. For when two "experts" disagree, how is one to choose? And how can one pose the right questions without already knowing some of the answer? But *Les Amis de la Terre* are pleased with it and plan others for the future. □

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NEWS AND VIEWS

Antarctic ice surges and theories of glaciation

from D.Q. Bowen

THEORIES of ice ages are never far from the limelight, but with the demonstration that the Earth's climate over the past 500,000 years has followed changes in the amount of solar radiation received at certain latitudes, as predicted by the Milankovitch model (Hays *et al. Science* **194,** 1121; 1976), the mystery seemed to be solved. But those authors would be among the first to admit that the model is really no more than a black box; a celestial mechanical input is followed by the output of a climatic signal. Although past behaviour of the climate can be deduced from oxygen isotope analyses of deep-sea cores, which allow inferences to be drawn concerning the interaction of the Earth's atmosphere, oceans and cryosphere, exactly how an ice age starts remains unknown. Any model should also explain not only how glaciation is initiated but also how it is brought to an end.

In 1964, A.T. Wilson (*Nature* **201,** 147) suggested that because the Antarctic ice-sheet is inherently unstable it would surge periodically into the southern ocean and form a huge ice-shelf. This would increase the Earth's albedo and the transfer of the consequent cooling to the northern hemisphere would lead to glaciation. Because the ice-shelf could not be sustained its eventual break-up would decrease albedo, heat would be transferred to the northern hemisphere and glaciation terminated. This was subsequently modelled and deemed eminently feasible climatologically (Flohn *Quaternary Res.* **12,** 135; 1979).

J.T. Hollin (*Nature* **208,** 12; 1965) pointed out that the global rise in sea-level after such a surge, possibly amounting to 15 m, should be capable of geological verification: that is, by evidence of a rapid, though short-lived, rise in sea-level at the end of interglacials. In 1977 (*Boreas* **6,** 33), he argued that it occurred at the end of oxygen isotope stage 5e, to initiate the Last Glaciation; but on page 649 of this issue of *Nature* he now suggests it was later, at the end of stage 5c. How is this shown? Unequivocal geological proof for such sea-level rises, and inferred surges of Antarctic ice, is difficult to obtain. How can a raised marine deposit be ascribed to such an event? In south-east England Hollin was able to show that some marine transgressions took place late in interglacials from the fact that their beach deposits overlie terrestrial ones dated by pollen analysis. Generally however, the rapidity of the postulated surge in sea-level, its brevity, and the incompleteness of the stratigraphic record, have conspired against a full and convincing demonstration. Nor is the surge likely to be recorded in the deep-sea cores, with their comparatively slow sedimentation rates, for it would have gone in less than 2,000 years — possibly considerably less.

Important new evidence bearing on the Wilson hypothesis comes from the Huon Peninsula, New Guinea, where a flight of uplifted coral terraces occurs more or less on the boundary between the West Pacific and Australian lithospheric plates. The old orthodoxy, which directed sea-level studies to stable coastlines (where, unfortunately, repetitive high sea-levels would have destroyed most pre-existing evidence), should now be received with caution, for the evidence is more effectively preserved in tectonically-rising areas. Provided that the tectonic factor can be separated from the depression caused in land level by the glacial masses of ice, which has been accomplished with considerable success in New Guinea (Chappell *Bull. Geol. Soc. Am.* **85,** 553; 1974) detailed records of sea levels are available at least for the past 130,000 years. Furthermore, the palaeoecology of the corals allows precise inferences to be drawn about rises and falls in sea-level. Unaltered aragonite coral may also be dated by $^{230}\mathrm{Th}/^{234}\mathrm{U}$ determinations. Thus much of the required critical data to test for sea-level surges is available.

P. Aharon, J. Chappell and W. Compston (page 669, this issue of *Nature*) show that the record is sufficiently good to establish, and date, sea-level fluctuations between 140,000 and 105,000 years ago. Stable isotope work on shells of the giant clam *Tridacna gigas* also provides information on the oxygen isotopic composition of the global ocean during this period. Because the clam lives in isotopic equilibrium with the ocean, a feature of some significance is an anomaly 120,000 years ago — during the Last Interglacial. This anomaly is interpreted as showing that water temperatures 120,000 years ago were cooler than at 130,000 years. Moreover the cooling took place within 1,000 and 2,000 years during a rise in sea-level of 8 m. Aharon *et al.* attribute this event to an Antarctic surge which caused sea-level to rise and transmitted cold water northwards. Thus the required geological test for Wilson's theory appears fulfilled. But did this event, 120,000 years ago, or the similar one identified by Hollin at ~ 95,000 years ago, initiate the Last Glaciation?

Evidence submitted in both papers is convincing; both use the relatively objective stratigraphic framework provided by the deep-sea oxygen isotope record. This, however, is concerned with chronostratigraphic 'Stages' — time-intervals defined from actual lithostratigraphic units (Shackleton & Opdyke *Quaternary Res.* **3,** 39; 1973) — and not with wholly inferred climatostratigraphic concepts such as 'glacials' and 'interglacials', or 'stadials' and 'interstadials'. It is quite possible that Antarctic surges occurred on several occasions not always related to interglacials as presently, and loosely, defined. In the period under consideration, a further difficulty arises from lack of agreement on how the end of the Last Interglacial should be defined. The classical Last Interglacial (Eemian) ended 120,000 years ago, but Woillard (*Quaternary Res.* **9,** 1; 1978) showed

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D.Q. Bowen is a Reader in the Department of Geography, University College of Wales, Aberystwyth.

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that two further interglacials occurred before the Last Glaciation commenced, 70,000 years ago. Definition rests on the classical north-west European pollen analytical demonstration of deciduous forest woodland. This somewhat parochial classification has been generally and uncritically accepted everywhere.

This vexed question is not new; volume 2 (No. 3) of *Quaternary Research* (1972), was devoted to the end of the 'Last Interglacial'. It is clearly desirable to agree on the definition of Global Standard Interglacials in relation to Stages objectively defined. In terms of the two contributions in this issue, neither of the coolings which their respective authors attribute to Antarctic surges led directly to the maximum build-up of ice during the 'Last Glaciation'. It is not impossible that such surges were the rule rather than the exception and that many abrupt temperature falls in the Pleistocene record are to be so explained. Perhaps major periods of continental glaciation only took place when a combination of Antarctice surge and favourable Milankovitch conditions arose. □

Osteology and prehistoric society

from Stephen Shennan

RECENT work on the chemical analysis of human bone from archaeological contexts confirms and extends the potential of human osteological studies for throwing light on man's social and economic development.

Archaeologists are increasingly concerned with explaining cultural change in terms of the social and economic processes at work within and between societies. One important advance has been in the use of evidence from excavated cemeteries to reconstruct past social organisation. Quantitative studies of the grave goods of dead individuals and the form and degree of elaboration of their burial monuments have been used to reconstruct the development of increasingly complex and differentiated societies as well as, in some cases, their devolution to more simple forms (for example Peebles *Mem. Soc. Amer. Archaeol.* **25**, 68; 1971; Rothschild, *Amer. Antiquity* **44**, 658; 1979).

Remarkably little attention has been given to the body itself however. Apart from its sex and age at death archaeologists have shown little interest in the information to be gained from the skeleton, and the obligatory physical anthropologist's report usually remains an unintegrated appendix. However, an increasing number of studies, particularly from North America, shows that the skeleton can yield information on aspects of diet and biological stress in past populations which are otherwise inaccessible and are vital to an understanding of cultural change.

In a recent paper Lambert, Szpunar and Buikstra (*Archaeometry* **21**, 115; 1979) describe the results of a chemical study of the skeletons of two burial populations from the eastern United States. The first belongs to the Middle Woodland period, about AD 400, when hunting and gathering provided a great variety of foods. The second belongs to the Late Woodland period, about AD 1200, when maize farming is well documented in the area. The bones were analysed by atomic absorption spectroscopy after sample preparation by a new method involving virtually complete dissolution of the bone ash. Particular attention was given to the problem of contamination of the bone by the surrounding soil and the conclusion was reached that although concentrations of iron, aluminium, manganese and potassium may largely reflect soil contamination, strontium, zinc, calcium, sodium and probably copper and magnesium give analyses that are representative of the bone.

The authors were able to investigate differences within as well as between each population. In the earlier one males and females had statistically identical bone compositions for every element examined, whereas in the later one there were significant differences, males having lower strontium and calcium and higher zinc concentrations, all diet-related elements. It seems that dietary differences between males and females developed which it is tempting to relate to the development of maize cultivation, and which may be associated with a greater differentiation of sex roles; the lower strontium concentration for males is at least consistent with a higher protein diet. Equally interesting, at the earlier site, where archaeological information indicated that the population could be divided into three groups of differing rank, it seemed that while the upper two groups did not differ significantly from one another in any elements, there were significant differences between the lowest group and a combination of the upper two in strontium, zinc, magnesium and sodium, again diet-related. Here the archaeological and analytical results together can show how the social distinctions marked in the burial rites correspond to dietary differences in life, although the nature of these differences is not yet clear.

In this example the need of the archaeologist for the physical anthropologist and chemist is clear enough but Buikstra (*op. cit.*) has also shown how the anthropologist can be misled if he fails to take archaeological considerations into account. The high frequency of pathologies in certain North American skeletal series from habitation sites of the Mid-Archaic period, has been ascribed to the rugged conditions of life. However, Buikstra was able to show that this phenomenon was a result of the nature of the sample: normal adults were buried in separate cemeteries but individuals not able to perform the range of 'normal' adult tasks were buried in the occupation area.

The potential of integrated work is further illustrated in the recent study by J. Lallo and J. Rose of another two prehistoric populations from eastern North America (*J. hum. Evol.* **8**, 323; 1979). They selected two populations from a single site in a period for which there is strong archaeological evidence of social and economic change, the first belonging to the Mississippian Acculturated Late Woodland period, AD 1050–1200, and the second to the Middle Mississippian, AD1200–1300. During this time society became increasingly less egalitarian, contacts became more widespread, and subsistence came to be more dependent on maize. Infectious lesions in bone were twice as common in the later period, as well as being more severe. Porotic hyperostosis, a condition of the bones of the skull reflecting increased bone marrow activity, was also more common. This condition has been observed elsewhere in prehistoric maize-eating groups (El Najjar *et al. Amer. J phys. Anthropol.* **44**, 477; 1976) and interpreted as reflecting iron deficiency. Various dental defects reflecting nutritional stress were also more common in the later period. Finally, examination of the distribution of age at death in the two populations showed a decrease in the life expectancy from 33 to 24.3 years. Lallo and Rose go on to offer a systematic explanation of these phenomena, indicating the way in which the social and economic changes independently documented lead to increasing disease and nutritional stress.

One hypothesis of this type has been put forward recently by R. Santley and E. Rose for the Basin of Mexico, one of the cradles of Mesoamerican civilisation (*World Archaeol.* **11**, 185; 1979). They argue that between 1500 BC and the Spanish conquest major periods of population growth correlated with periods of balanced nutrition, whereas phases of population stability corresponded to times of nutritional shortages and stress. They suggest that in hard times the elite urban populations would have used their power to ensure an adequate nutritional base for themselves at the expense of the rural population. Whether or not their idea proves correct, its testing will bring

Stephen J. Shennan is in the Department of Archaeology, University of Southampton.

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osteological analysis of the type described above into the explanation of some of the major developments in New World prehistory.

All these studies show the importance of using the osteological evidence to make inferences about the human biological changes which have accompanied social and economic development, and which not only tell us more about those developments but may themselves have been responsible for them in certain circumstances, providing one of the 'kicks' which set positive feedback processes in motion. □

Human teratocarcinoma workshop

Clinicians and mammalian embryologists are launching a concerted attack on human teratocarcinoma. The embryologists are moving on to this new challenge after some years spent manipulating mouse teratocarcinoma stem cell lines, obtained originally from tumours of the murine gonads. The value of such cell lines lies in the ease with which they can be induced to differentiate in a manner thought to be analogous to certain events of natural development. Although mouse development could hardly be said to be understood yet, insights into human development are now hoped for.

But embryologists are faced with the usual problems of working with human material. It is difficult to obtain, requiring the cooperation of surgeons who will ensure that a reasonable specimen is put aside before the whole tumour is claimed by a pathologist and doused in formalin. The problems of pathology and nomenclature are also formidable, and participants at a European workshop on human teratocarcinoma at Imperial Cancer Research Fund Laboratories (ICRF) in London on 28 January were not always sure what they were talking about. Nor were they all particularly confident of the difference between teratocarcinoma, seminoma and yolk sac tumour, although all three originate in the same anatomical region.

In future they will cooperate in obtaining cell lines and in characterising those already established. The workshop will gather again in the autum. Meanwhile information about the cell lines, their biochemical and immunological characteristics, is to be collected by Brigid Hogan of ICRF's Mill Hill laboratories for subsequent distribution. Later there may be a human teratocarcinoma newsletter.

M.L.

Sails set for the myosin active site

from Peter Chantler

WHEREAS kinetic secrets are being prized almost daily from the myosin and actomyosin ATPase mechanisms knowledge of their structural aspects remains in the dark ages. The quest for structural information on the active site of myosin has been bedevilled by false leads. One example is that of the two thiol groups (SH-1 and SH-2) on each myosin head which were originally considered essential to the myosin ATPase mechanism, possibly being intimately involved with nucleotide at the active site. More recent studies however, have thrown doubt on the idea that these thiol groups are directly involved in the active site and a new twist to the story has recently emerged from Yount's laboratory at Washington State University. By an innovative technique in which the fate of radiolabelled nucleotide substrates was followed during chemical modification of myosin with cobalt, they have clarified some aspects of the relation of the thiol groups to the true active site and at last opened up a way for its unambiguous definition (Wells *et al. Biochemistry* **18**, 4793; 4800; 1979; Wells & Yount *Proc. natn. Acad. Sci. U.S.A.* **76**, 4966; 1979).

Taking advantage of the exchange characteristics of Co (II) and Co (III) coordination complexes, myosin subfragment 1 (S-1) was exposed to a mixture of $\mathrm{CoCl_2}$, 1,10 phenanthroline and $[\mathrm{Co^{III}}\ (\mathrm{phen})_2\mathrm{CO_3}]^+$ with a resultant inactivation of the ATPase activity (Wells *et al. Biochemistry op. cit.)* caused by tight chelation of one cobalt ion per mole S-1 which occurred in parallel with the loss of two sulphydryl groups. The mechanism proceeds by way of a S-1.$\mathrm{Co^{II}}\ (\mathrm{phen})_x$ intermediate ($\mathrm{Co^{II}}$ freely exchangeable) which undergoes exchange with $[\mathrm{Co^{III}}\ (\mathrm{phen})_2\mathrm{CO_3}]^+$ to yield a S-1. $\mathrm{Co^{III}}\ (\mathrm{phen})_x$ compound ($\mathrm{Co^{III}}$ not exchangeable). Such *in situ* oxidation of the bound cobalt is extremely mild; indeed, the S-1.$\mathrm{Co^{III}}\ (\mathrm{phen})_x$ can be reduced by short incubation with reagents such as $\mathrm{Fe^{II}}$EDTA which fully restore enzymic activity. The simultaneous loss of $\mathrm{NH_4}^+$-EDTA, Ca^+ and Mg^+-ATPase activities on cobalt binding and the blocking of the binding by *p*-N,N′-phenylenediamine (which crosslinks SH-1 to SH-2) identified the two sulphydryls lost as SH-1 and SH-2 and showed that these two thiols could be cross-linked by the cobalt and therefore approach to within 3-5Å of each other (the distance depending on whether there was *cis* or *trans* chelation).

When the fate of ^{14}C-labelled nucleotides was followed during the course of Co modification, inactivation of the ATPase amazingly paralleled incorporation of nucleotide so that complete inactivation occurred on simultaneous binding of one gram-atom of cobalt and one mole of nucleotide per mole S-1. This not only directly demonstrates the separate identities of the active site and the SH-1 and SH-2 thiols but also shows, consistent with other evidence, the interdependence of the thiol and active sites. When cobalt chelates SH-1 and SH-2, nucleotide at the active site is prevented from leaving; when nucleotide binds to the active site, cobalt chelates SH-1 and SH-2 much more easily. In addition, a new phrase enters the textbooks: active site trapping — a technique, in serendipitous cases, as here, of great potential power. When the experiment was performed in the presence of MgATP the trapped nucleotide always manifested itself as MgADP. Although the relationship between cobalt modification and nucleotide hydrolysis is unclear it is apparent from the relative rates of the two reactions that the trapped ADP did not originate from a rebinding of previously hydrolysed substrate.

As active site trapping seems to be equally effective in the presence of adenosine 5′-(β,γ-imido)-triphosphate, a non-hydrolysable analogue of ATP, the technique may well offer some insight into mechanism. But its real future lies in conjunction with photoaffinity probes such as 8-azidoadenosine triphosphate which the authors have also trapped (Wells & Yount *op. cit.*). The power of the photoaffinity probe, in more conventional mode, has been seen recently in the binding and incorporation of arylazido-β-alanine-ATP into proteolytic subfragments of myosin followed by tryptic proteolysis (Szilagyi *et al. Biochem. biophys. Res. Commun.* **87**, 936; 1979). The reagent labelled an N-terminal 25K peptide of myosin; there was no incorporation into the fragment containing SH-1 and SH-2. As this latter fragment is separated from the 25K peptide by a 50K peptide (Szilagyi *et al. op. cit.*) it is apparent that the sequentially remote 'essential' thiol region must be juxtaposed near to the active site in the tertiary structure of the enzyme.

Detailed speculations on which amino acids are involved at the active site of myosin are unwarranted but the most likely residue seems to be arginine (Mornet *et al. Eur. J. Biochem.* **100**, 421; 1979), found at the heart of many nucleotide-binding enzymes (Riordan *et al. Science* **195**, 884; 1977). Tryptophan, on the cards for many years becuase of the nucleotide-dependent tryptophan fluorescence enhancement of skeletal muscle myosin (Werber *et al. Biochemistry*, **11**, 2872; 1972) seems less likely now in view of the absence of such effects in invertebrate myosins (Chantler & Szent-Gyorgyi *Biochemistry* **17**, 5440; 1978). Lysine, histidine and tyrosine have also been proposed. At last the opportunity to find out for certain is here. □

Peter Chantler is at the Imperial Cancer Research Fund Laboratories, Lincoln's Inn, London.

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Ribonuclease still under attack

from Barry Robson

THE enzyme ribonuclease has always been popular with experimentalists seeking to understand the relations between chemical formulae, three-dimensional structures, and functions of protein molecules. Ribonuclease is a particularly attractive weak spot in nature's defences because it was the first enzyme to have its chemical formula determined (as a sequence of amino acid residues), one of the earliest enzymes to have its three-dimensional structure elucidated, and the first protein for which it was carefully demonstrated that the chemical formula alone determines the three-dimensional structure and function. It continues to be a prime target in a number of recent investigations.

One important approach to understanding the relationship between the chemical formula and the final structure is to attempt to follow how a protein molecule folds up after it has been unfolded. In particular, it would be of interest to know how a protein folds up so rapidly: the current theory is that it is rapidly directed towards the native structure by a process of nucleation in which certain structural features such as α-helices and β-pleated sheets form first, propagating further structure around them. By tritiating the exchangeable amide protons of unfolded RNase A and following exchange of tritium with the surrounding solvent during refolding, Schmid and Baldwin (*J. molec. Biol.* **135**, 199; 1979) have been able to confirm that the protein does fold up by a stepwise process, early stages being hydrogen bonded structures in which the tritium is protected from exchange. Since α-helices and β-pleated sheets are such structures, this would seem at first consideration to support the nucleation model. In particular, it supports the idea that such intermediate structures are metastable, possibly in appropriate conditions stable, and form relatively rapidly, as suggested previously by studies on penicillinase using more standard techniques (Robson & Pain *Biochem. J.* **155**, 325; 1976).

However, the nucleation idea is complicated by the experimental observations that the stability of the intermediate structure depends on interactions with the rest of the protein. In their case Schmid and Baldwin find that an artificially cleaved-off fragment of ribonuclease known as the S-peptide seems to be essential for the stability of the intermediates. In a further detailed study Labhardt and Baldwin (*J. molec. Biol.* **135**, 231 and 245; 1979) observed (1) that S protein (ribonuclease A without S-peptide) folds up some hundred times more slowly than intact ribonuclease A, and (2) that on adding S-peptide to S-protein, the combination of S-peptide and S-protein occurred at an intermediate stage, rather than at the end of the folding process. Labhardt and Baldwin exclude the model for protein folding in which there is an initial slow diffusion of the molecular conformation into a restricted range of three-dimensional structures, and demonstrate instead successive folding stages with rates dependent on the stabilities of intermediates from preceding steps. Whether this supports or excludes the classical model for nucleation depends on how one interprets that model. Rightly or wrongly, many workers seem to associate the idea of nucleation with a key directing step, the question of rates being one of their own preference. That Labhardt and Baldwin are more proper about the definition of nucleation is, however, irrelevant, since they provide a self-consistent and meaningful description of the folding process.

Baldwin and colleagues also give considerable attention to the fast and slow folding species of RNase in their studies. In this they accept the current interpretation that the slower species is the product of a kind of isomerisation of proline residues in the unfolded form. This may, however, be a case in which attention to one enzyme is less satisfactory. The interpretation in terms of isomerisation is best tested by reference to the different rates of folding kinetics of many different proteins, correlating the proline content with those rates (Stellwagen *J. molec. Biol.* **135**, 217; 1979).

Studies of this type provide a logical framework for the theoreticians, whose principal goal is to predict the three-dimensional structure of a protein from its amino acid sequence alone, and ultimately to design new proteins by chosing the amino acid sequence to give the required conformation and function. A major difficulty here is that many amino acid residues considered alone have only a subtle effect on the overall conformation of the molecule: all the amino acid residues tend to interact in a complex way. The theoretician is faced with having to handle calculations concerning a delicate balance of forces and it is doubtful whether his armoury is as yet adequate to this task. Gutte, Daumingen and Wittscheiber (*Nature* **281**, 650; 1979) recently mounted an audacious rear attack on this problem by designing an artificial RNase of 34 residues, having chosen amino acid residues and their sequence which would seem likely to be a very strong generator of the conformation required. Particular emphasis was given to the strong tendencies of certain residues to form α-helix or β-pleated sheet. The philosophy was that the more subtle and ambiguous aspects should be avoided, and this rationale seems justified by their subsequent synthesis and investigation of the protein. Briefly, although a detailed analysis of the three-dimensional structure was not possible, the protein indeed had significant activity, particularly when in a dimerised form.

More precisely, one should say that the production of RNase activity may have been something of a bonus, since they state that their immediate aim was to produce a protein with nucleic acid binding ability. This is a necessary but not sufficient condition for RNase activity and it is conceivable that catalytic activity might have to have been introduced at a later stage by further refinement of the sequence. This turned out not to be the case, and it seems that their work brings the status of the theoretician's art one step nearer to practical application. However, noting that the activity of the artificial RNase was only a few per cent of natural RNase, one should consider appropriate control experiments. As Gutte *et al.* discussed, random amino acid sequences often have significant enzymic activities of various types. Perhaps it would have been helpful to compare the activity of their artificial RNase with that of random amino acid sequences of similar length and composition to establish the importance of specific consequences. If the histidine residue was responsible for the activity, then the precise environment may have been rather less important. Further, catalytic activity of another kind has been associated with a mere 10-residue sequence (Chakravarty *et al. Experientia* **29**, 786; 1973), so it does not follow that all of the artificial molecule was in the expected conformation. Particularly when the substrate is a macromolecule, this may itself be a source of the appropriate environment and conformational stability, involving a kind of 'induced fit' of the synthetic protein.

RNase and their relatives are among some of the smallest enzymes known and their ability to interact with nucleic acid makes them useful models both for modern and prebiotic systems of more general interest. Undoubtedly the recent studies will encourage the continued use of RNase and its synthetic analogues. □

Barry Robson is in the Department of Biochemistry, University of Manchester.

Correction

In the article 'Probing the insulin receptor' (*News & Views* **282**, 11; 79) the incorrect impression may have inadvertently been given that the photolabelled probes used by C.C. Yip *et al.* were less well characterised than those of other groups. (line 4, paragraph 3, page 11). The probes are in fact specifically photolabelled at the B-29, A1-B29 or B1 positions, which was not stated in the article.

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Post-receptoral processes in colour vision

from J.D. Mollon

ALTHOUGH our subjective experience of hue may seem endlessly varied, the process of colour vision begins with just three classes of retinal receptor, each sensitive to a large part of the visible spectrum. Long before these delicate structures could be directly measured by physiologists, the limitation to three parallel channels was deduced by psychophysicists — those who work out the logic of sensory systems from the responses of conscious observers to systematically varied inputs. More difficult for the psychophysicist was the task of discovering how absorption varied with wavelength for the individual classes of receptor, since these 'spectral sensitivities' overlap substantially and it is not possible to isolate, say, the short-wavelength receptors simply by stimulating the retina with violet light. However, experimental stratagems were developed to cause an observer's response to depend on signals from only one class of receptor: for example, the psychophysicist might use monochromatic adapting stimuli to suppress two of the three classes or he might choose spatial and temporal parameters for his target stimulus that were suspected to favour one class of receptor or he might seek observers genetically lacking either one or two of the three classes. For many decades, psychophysical estimates of the receptoral sensitivities differed widely, but the past 20 years have seen a quiet convergence on the wavelengths 440 nm (violet), 535 nm (green) and 565 nm (yellow green) for the peak sensitivities of the three classes; and although these values need correction for selective absorption in the media of the eye and although second-order disagreements remain, the psychophysical estimates are in first-order agreement with very recent physical measurements of individual receptors (see papers by Bowmaker and collaborators in *J. Physiol. Lond.*, **298**, 1980).

Isolating opponent channels

It is generally held that a second kind of parallelism follows the receptor stage. There are some channels (loosely called 'luminance' or 'non-opponent' channels and probably needing further subdivision) that receive signals of the same sign from the receptors with peak sensitivities at 535 and 565 nm, and there are others (the 'colour-opponent' channels) that receive antagonistic signals from different classes of receptor. The colour-opponent channels extract information about wavelength by comparing the relative absorption in different classes of receptor. (The receptors themselves, of course, are individually colour-blind, since they are broadly tuned and since a photon, once absorbed, yields the same signal whatever the wavelength of the stimulating light.)

Psychophysicists have developed stratagems to isolate individual channels at this second stage and several experimental techniques for isolating opponent channels became explicit at a meeting on post-receptoral processes held in London on 9 January by The Colour Group, an inter-disciplinary society linking those with physical, physiological, and aesthetic approaches to colour.

If detection thresholds are measured for weak monochromatic stimuli presented on various steady adapting fields, detection by opponent channels is favoured if the targets are of long duration or large area — presumably because these channels integrate in time and space more than do (some) non-opponent channels. Evidence for this position was presented at the meeting by E. King-Smith (University of Manchester) and by D.H. Foster (University of Keele).

King-Smith suggested that strong, white-adapting fields will adapt non-opponent more than opponent channels, because the sensitivity of the latter is controlled by the difference in signals from different receptors and so will change little with absolute light intensity. J. Mollon (Cambridge University) added the complementary suggestion that strongly coloured, monochromatic fields (as used classically by Stiles) cause detection to depend largely on non-opponent channels, because such fields disproportionately desensitise opponent channels.

Foster described a new technique for isolating opponent channels. On a standard adapting field is superimposed a small, steady 'auxiliary' field, which is congruent with the target flash. If, say, the target is of long wavelength and only the main adapting field is present, the field is most effective in reducing sensitivity when its wavelength is ~570 nm; introduction of the auxiliary field shifts the wavelength of maximal field sensitivity to ~610 nm, suggesting that detection now depends on a channel which receives excitatory signals from the 565-nm receptors and antagonistic signals from the 535-nm receptors. The effect of the auxiliary field is abolished if the auxiliary field is significantly larger than the target spot. Presumably the presence of a contour coincident with that of the target masks a spatial transient that would otherwise allow detection by a non-opponent channel.

Mollon proposed that detection will depend only on an opponent channel if the input signal is confined to the violet-sensitive receptors. Perhaps because the short-wavelength component of the retinal image is usually out of focus (owing to the chromatic aberration of the eye), signals from the violet-sensitive receptors seem to be used only to provide information about hue. Because these receptors are far removed in peak sensitivity from the other two classes, the sensitivity of the opponent channel can be modulated with long-wavelength fields without significantly changing the rate of photon absorptions in the violet-sensitive receptors themselves — a great experimental convenience.

According to a hypothesis of Pugh and Mollon (*Vision Res* **19**,293; 1979), an opponent channel is most sensitive to input perturbations when it is near the middle of its operating range. In collaboration with P.G. Polden and A. Stockman, Mollon has found large oscillations in sensitivity to violet targets during adaptation to a bright yellow field; these are thought to arise from changes in the balance of inputs to the opponent channel during bleaching of the long-wavelength receptors. King-Smith reported that in several ocular diseases (for example, retrobulbar neuritis and glaucoma) patients show a greater loss of sensitivity to short-wavelength targets when the eye is adapted to a yellow field than when the adapting field is white and relating this finding to the above hypothesis proposes that the operating range of opponent channels is reduced in disease.

It is well established that adaptation to a visually presented grating will briefly raise the threshold for a subsequently presented 'test' grating of similar orientation and spatial frequency. K. Ruddock (Imperial College, London) has previously reported that if the adapting and test gratings are presented to one eye, a low-frequency spatial pattern presented to the other eye during adaptation will reduce the extent of adaptation. V. Waterfield and he have now shown that the effectiveness of this contralateral stimulus depends on its wavelength. Moreover, variation of its actual pattern intriguingly reveals channels of different spectral sensitivities: thus a simple grating gives the photopic luminosity function, a pattern of blobs reveals a long-wavelength channel with opponent inputs from the 565-nm and 535-nm receptors, and the negative of the latter pattern gives a green-sensitive mechanism with a small inhibitory long-wavelength input.

Two possible pitfalls

Many papers in the recent literature have advanced a further stratagem for isolating colour-opponent channels: the observer is required to detect a spatial or temporal transition between stimuli that are of different wavelength but equal luminance. However, I feel that it is unlikely that this device will safely eliminate detection by 'luminance' channels. Suppose that the target is a red field briefly substituted for a white field of equal luminance. At the onset of the red field, an increment is presented to the 565-nm receptors and a decrement to the 535-nm receptors. We are

J.D. Mollon is at The Psychological Laboratory, University of Cambridge.

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obliged to adopt an assumption for which there is no proof, that the transient responses generated in the two classes of receptor will exactly cancel out in all the putative 'luminance' channels; we must suppose that the weightings of inputs from the different receptors are in a constant ratio for all channels and that the transient waveforms of different signs are symmetrical. Transients in space and time are biologically significant and it would be odd if the retina allowed them to be lost in this way.

A second pitfall may lie in relating the detection measurements mentioned above directly to the classical phenomenological measurements (see for example Hurvich & Jameson *Psychol. Rev.* **64,** 384; 1957) that kept alive the concept of opponent processes during a period of neglect. General understanding of the first stage of colour vision was earlier held back by a widespread but unjustified belief that the peak sensitivities of the three receptors should correspond to phenomenologically primary colours, red, green and blue; it may equally be a mistake to suppose now that the opponent processes of the retina must be directly related to the phenomenological oppositions of blue and yellow and of red and green. S. Zeki (University College, London) presented his measurements of the spectral sensitivities of individual nerve cells in Area V4 of the cerebral cortex of the rhesus monkey; even at this central level, although many cells have narrow bandwidths (10–20 nm), the peak sensitivities do not cluster very tightly at the four phenomenologically pure hues, blue, yellow, green and red, but are distributed throughout the spectrum, with possibly a gap at 560–570 nm.

Colour constancy

As we view an object from different distances and angles or under different illuminations, our perception changes much less than does our retinal image. This stability of our perception is known as perceptual constancy. It is an everyday observation that a sheet of white paper looks equally white in daylight and in the yellowish illumination of domestic tungsten light, although if outdoor film were used to photograph the paper indoors, the resulting transparency would have a strong yellow cast. The correction achieved by our visual system (but not by the photographic process) is called colour constancy. One of the most interesting results presented at the meeting was Zeki's finding that some individual cortical cells show colour constancy: if an array consisting of many different coloured patches is placed before a monkey, and, say, a green patch is arranged to cover the receptive field of a green-sensitive cell, the cell responds to the patch despite gross changes in the spectral composition of the illumination falling on the array; but the cell does not respond if, say, a red patch is placed in its receptive field and the overall illumination is adjusted so that the spectral composition of the flux reflected from the red patch was identical to the flux previously reflected from a green patch to which it did respond (Zeki *Nature* in the press). Under the latter condition, of course, the patch looks red, not green, to a human observer (see Land *Sci. Amer.* **237,** December; 1977) just as, in the classical demonstration of size constancy scaling, an after-image of fixed retinal dimensions looks of different sizes when projected on surfaces at different distances.

Colour preferences

The layman reasonably expects the colour scientist to be much concerned with people's colour preferences, but the experimental study of colour preference has an unhappy history of inconsistent results and has been neglected in recent years. Reviewing this field for the meeting, I.C. McManus (Bedford College and St. Mary's Hospital, London) noted that earlier studies had confounded variation in hue with variations in saturation and lightness, and he described a study, performed in Cambridge in collaboration with A. Jones and J. Cottrell, in which this failure was corrected. Another feature of this new study was the use of the 'method of paired comparisons': each of a large set of coloured chips was paired with every other chip and the subject was asked to express a preference between the members of each possible pair, thus allowing the experimenter to derive a measure of internal consistency. When saturation and lightness are held constant, the population shows a clear preference for blue and a clear dislike of yellow. Further analysis shows differences between individuals but consistency within an individual's data; differences between individuals seem to be almost entirely due to differences in lightness preference and saturation preference, with very small inter-subject differences in hue preference. ☐

100 years ago

THE CRAYFISH[1]

Common and lowly as most may think the crayfish, it is yet so full of wonders that the greatest naturalist may be puzzled to give a clear account of it." These words from von Rosenhof, who in 1755 contributed his share to our knowledge of the animal in question, are cited by Prof. Huxley in the preface to the careful account of the English crayfish and its immediate congeners, which forms the latest volume of the International Scientific Series. The book is not designed for "general readers," those somewhat luxurious but presumably intelligent persons for whom so much scientific knowledge is chopped and spiced at the present day. It is, as we gather from the author's statement, intended as an introduction to serious zoological study, for those who will turn over its pages, crayfish in hand, and carefully verify its statements as to details of structure with scalpel and microscope. To these and also to those who are already well versed in crustacean anatomy, the book will have great value and interest; to the latter more especially, as showing how in the careful study of one organism we are "brought face to face with all the great zoological questions which excite so lively an interest at the present day," and as an exhibition of that "method by which alone we can hope to attain to satisfactory answers of these questions."

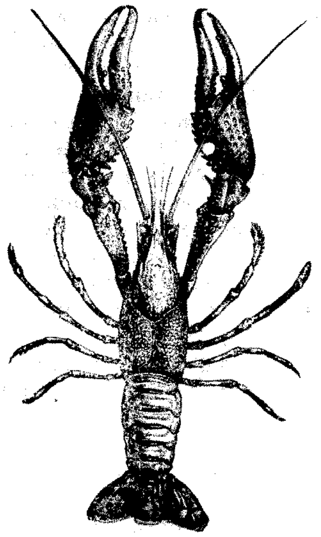

Fig. 4.

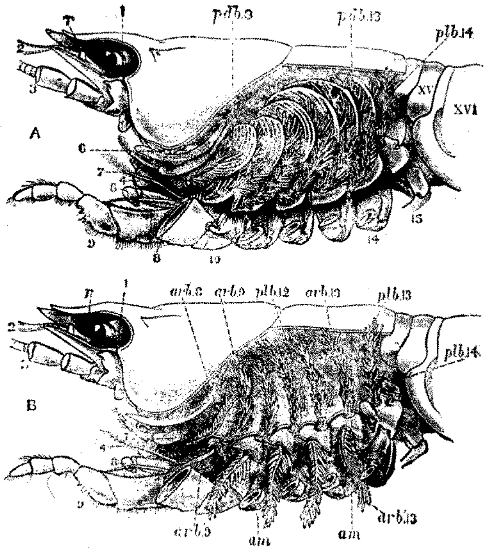

Fig. 5.

From *Nature* **21,** 12 Feb., 353; 1880.

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[1] "The Crayfish: an Introduction to the Study of Zoology." By T.H. Huxley, F.R.S. (London: Kegan Paul, 1880.)

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REVIEW ARTICLE

Cellular site of opiate dependence

H. O. J. Collier

Miles Laboratories Limited, Stoke Poges, Slough SL2 4LY, UK

Experiments on whole animals, guinea pig isolated ileum, individual neurones in situ *and cultured neuroblastoma–glioma hybrid cells indicate that opiate dependence and associated tolerance develop within neurones bearing specific opiate receptors (opiate-sensitive neurones). The essential change appears to be an hypertrophy of the cyclic AMP system, in response to inhibition by opiate of a neuronal adenylate cyclase.*

CHRONIC treatment, either continuous or intermittent, of a mammal with an opiate drug produces tolerance, indicated by a lowered responsiveness to the drug, coupled with a dependence characterised by a heightened responsiveness to opiate antagonists and disturbance on withdrawal of drug. A basic requirement for understanding the molecular mechanism of this remarkable phenomenon is to identify the cellular site at which it happens. As long as the phenomenon was studied only in whole animals, as it was with few exceptions until quite recently, there was little opportunity of identifying its site, although some experiments pointed towards it. The recent development of methods of studying opiate dependence and tolerance in the myenteric plexus of guinea pig ileum, in individual neurones of the nervous system *in situ* and in cultures of cells of neural origin has made it possible to identify the main cellular site at which opiate dependence and associated tolerance occur. This article presents and discusses implications of the evidence that this phenomenon occurs within the opiate-sensitive neurone. In this discussion the term 'opioid' will be used to include both opiates, which are analgesic derivatives of opium, such as morphine, and synthetic or endogenous substances having similar actions.

Opiate-sensitive neurone

The opiate-sensitive neurone is one that possesses specific opiate receptors, of any variety. It can be characterised in three ways. First, interaction between opiate molecules and these receptors produces a change in the neurone that results in the agonist actions of the drug. Second, this interaction between opiate and receptor can be blocked by the pure opiate antagonist, naloxone[1]. Third, these actions are stereospecific, or perhaps more correctly, stereoselective, in that they are exerted by small does of only the (−) enantiomers of opiates or opiate antagonists.

Such actions of opiates contrast with their nonspecific actions, which are not antagonised by, and may be shared with, naloxone. Thus, both morphine and naloxone, being phenols, can act as cofactors for prostaglandin synthetase[2]. Again, both drugs inhibit hyperalgesia of rat paw elicited by intraplantar injection of prostaglandin E_2 (ref. 3). Non-stereospecific actions are produced by both the (+) and (−) enantiomers of opiates; for example, the same behavioural excitation is elicited by injecting either (+) or (−) morphine into the periaqueductal grey matter of rat brain[4]. This stimulatory action is not blocked by naloxone and is therefore also nonspecific.

Opiate-sensitive neurones exist in both the central nervous system (CNS) and the myenteric plexus. In the CNS, the action of opiates on the majority of these neurones is inhibitory; but a minority appears to be excited by opiates. For example, in a recent survey of the responses of individual neurones *in situ* in different areas of the rat brain[5], the opiate drug, normorphine, and the opioid peptides, β-endorphin and Met-enkephalin, inhibited nerve impulse production in the majority of responsive cells in the brainstem, cerebral cortex, caudate nucleus and thalamus; whereas each substance excited the hippocampal pyramidal cells. In the myenteric plexus, opiates inhibit the cholinergic neurone supplying the longitudinal muscle of the guinea pig ileum[6,7] whereas they stimulate a neurone supplying the circular muscle of the rat colon[8,9].

Tolerance or dependence is usually observed towards the specific inhibitory actions of opioids; but it has also been described towards their specific stimulatory actions, both in the CNS[10,11] and in the myenteric plexus[8,9]. Since the above experiments on pyramidal hippocampal cells were reported, however, evidence has been obtained that their apparently specific stimulation by opioid is in reality a disinhibition of the adjoining basket cell, which itself maintains a tonic inhibition of the pyramidal cell[12]. Possibly the other specific stimulatory actions of opioids also may eventually prove to be disinhibitions. Because of this, and because of the greater wealth of experimental data, only dependence and associated tolerance towards the inhibitory actions of opioids will be discussed below.

Before we enter this discussion, two principles should be noted. First, the response to binding of opiate molecules with specific receptors occurs at two rates–acute and subacute (or chronic). The acute response to a single dose may be assumed to begin immediately after the administered opiate has reached and bound with its receptor. The duration of the acute response can be measured in minutes. The subacute response to continued treatment, expressed as tolerance and dependence, is apparent within hours of treatment beginning. The duration of the subacute response has a briefer component, measurable in hours, and a longer component, measurable in days[13,14]. Very prolonged effects of chronic treatment have also been observed, but they are not included in the present discussion. Second, in experiments on tolerance and dependence, a fundamental principle of uncertainty operates[15]. Tolerance cannot be measured directly without administering drug, nor dependence without withdrawing it. Hence we cannot measure tolerance and dependence in the same preparation at the same time. Measurement of tolerance and dependence separately in parallel preparations, however, has led to general agreement that opiate dependence is accompanied by tolerance, although the extent of the decrease in responsiveness to opiate (tolerance) may be numerically less than that of the increase in responsiveness to opiate antagonist (dependence)[16].

Experiments in whole animals

Attempts to determine whether neurotransmitters were intimately involved in the mechanism of opiate dependence provided probably the earliest evidence on its cellular site, although their meaning was not clear to us at the time. In 1972, we showed that, in the dependent rat, naloxone elicited 10 behavioural signs of precipitated withdrawal, all of which were lessened or abolished by giving heroin shortly before naloxone

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challenge[17] (Table 1). In contrast to heroin, substances such as atropine, indomethacin and *para*-chlorophenylalanine, that block the action or formation of putative messengers between neurones, inhibited some signs of abstinence, intensified others and left still other signs unaffected. For example, atropine, given 30 min before withdrawal was precipitated by naloxone, significantly decreased the incidence of jumping, diarrhoea and chewing, but significantly increased that of irritability to touch and paw tremor, and left unchanged the incidence of teeth chattering, ptosis, head shakes and body shakes (Fig. 1). Essentially similar results were obtained when the withdrawal syndrome was modified with an inhibitor of the synthesis of serotonin or of prostaglandins. A simple and attractive explanation of these observations is that dependence in the whole animal occurs within neurones of the CNS that bear specific opiate receptors, and that other neurones with which these communicate are only secondarily affected.

Naloxone inhibits the induction of dependence, indicating that induction is specific, although a relatively high dose is needed[18]. That sensitivity to naloxone greatly increases in dependence[16,17,19] also accords with dependence occurring within the opiate-sensitive neurone, since the amount of naloxone required to precipitate withdrawal in dependent states is so low that the naloxone is presumably acting only at the opiate receptors on the neurones.

Table 1 Blockade by heroin, given before challenge, of morphine withdrawal signs elicited by naloxone challenge in dependent rats[17]

Withdrawal sign	No. of rats positive Saline	Heroin	Significance (P value)
Jumping	8	0	<0.001
Teeth chattering	10	1	<0.001
Irritable to touch	5	0	<0.02
Irritable to handling	10	1	<0.001
Diarrhoea	12	0	<0.001
Chewing	12	0	<0.001
Ptosis	11	4	<0.005
Body shakes	2	0	=0.24
Head shakes	5	1	=0.08
Paw tremor	3	0	=0.11

Twenty-four rats were made dependent by subcutaneous (s.c.) injection of 150 mg per kg of a sustained-release preparation of morphine; 23.5 h after this injection, 12 rats received heroin 40 mg per kg s.c. and 12 received saline; all rats were challenged 30 min later with naloxone 1.0 mg per kg s.c. Observers of the rat behaviour were unaware of what treatment each animal had received.

Myenteric plexus of guinea pig ileum

In the isolated ileum of the guinea pig, opiates inhibit contraction of the longitudinal muscle elicited by electrical stimulation of the post-ganglionic cholinergic neurone[1,6,7]. Continued exposure to opiate of the isolated ileum at 4 °C, 22 °C or 37 °C produces tolerance to this effect[7,20–23], and dependence, usually expressed as a contracture of the longitudinal muscle on challenge with naloxone[21–26]. As in the whole animal, tolerance and dependence thus induced in the isolated ileum are specific, in that induction is inhibited by naloxone[22,26]. Effects in the ileum are also stereospecific, in that tolerance and dependence are induced by the (−) but not by the (+) enantiomer of a pair of stereoisomeric opiates and that withdrawal contracture is elicited by (−) but not by (+) naloxone[22–26].

Three properties of dependence in the isolated ileum point towards its site. First, that the withdrawal contracture is blocked by hyoscine or atropine[21,23,26] places dependence within the myenteric plexus of neurones rather than in the longitudinal muscle. Second, that the ganglionic blocking drug, hexamethonium does not prevent the induction or expression of dependence[22,23,26] places dependence in the post-ganglionic region of the plexus, in which the opiate-sensitive neurones are present. Third, that responsiveness to naloxone increases both with duration of exposure to opiate and concentration of opiate used[26], until a very low concentration of naloxone is effective (Fig. 2), suggests, as already indicated, that the opiate-sensitive neurone is itself the site of dependence.

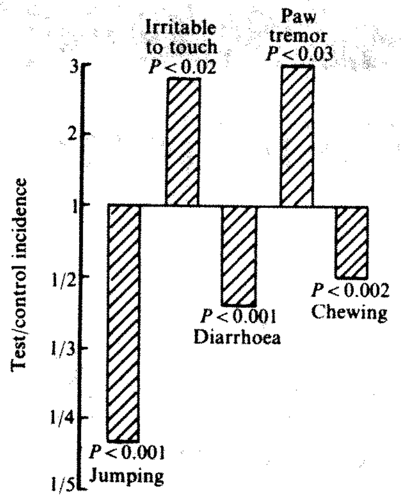

Fig. 1 Action of atropine on incidence of different withdrawal effects in morphine-dependent rats[17]. Withdrawal was precipitated by naloxone (1.0 mg per kg s.c.) 24 h after injection of a sustained-release suspension of morphine (150 mg per kg s.c.). Atropine (40 mg per kg s.c.) or 0.9% NaCl in water was given 30 min before naloxone. There were 32 test animals and 32 controls. The test/control incidence is the ratio of the incidence of the withdrawal effect in test and control groups. Atropine given before naloxone did not significantly change the incidence of teeth chattering, ptosis, body shakes or head shakes (Table 1).

Individual neurones *in situ*

Opiate tolerance and dependence have been studied in individual neurones *in situ*, in the sensorimotor cortex of the rat brain[27] and in the myenteric plexus of the guinea pig ileum[23]. In these studies, the action currents of nerve impulses were recorded by microelectrodes and drugs were applied by iontophoresis through micropipettes. Since neurones were used in which opiates inhibited the generation of nerve impulses and naloxone blocked the effect of opiates, these studies give direct information on tolerance and dependence in opiate-sensitive neurones.

In the sensorimotor cortex, continuous exposure to morphine for 40 min induced insensitivity to morphine, followed by a state of spontaneous excitation in the neurone that seems to represent dependence (Fig. 3). The inhibitory effect of morphine was also tested on cortical neurones stimulated with L-glutamate or acetylcholine in opiate-naive and opiate-dependent rats. Whereas 63 of 83 cortical cells tested were inhibited by morphine in the opiate-naive animals, only 1 in 50 was so inhibited in the dependent rats[27]. Thus, there is a high probability that tolerance occurs in the neurones that are normally inhibited by morphine. Since these tolerant neurones became excited when treated with naloxone, we can infer that they were dependent also.

Table 2 Cyclic AMP production by NG 108-15 cells cultured for 48 h in the absence or presence of morphine (10 μM)

Challenge solution	Cyclic AMP produced by cells cultured Without morphine	With morphine
Control	21	23
Naloxone	21	37
PGE$_1$	264	81
PGE$_1$ + naloxone	241	1,183
Adenosine	103	65
Adenosine + naloxone	72	217

After 48 h incubation, cells were challenged by incubation for a further 10 min with addition of 10 μM naloxone, PGE$_1$ or adenosine, as indicated. Cyclic AMP was then extracted and estimated in pmol per mg protein. Data from ref. 29.

In the myenteric plexus of guinea pig ileum, post-ganglionic neurones were made tolerant and dependent by incubation with morphine, normorphine or levorphanol for 24 h at room temperature, in the presence of hexamethonium and hyoscine[23]. In such neurones, administration of normorphine by microiontophoresis much less readily inhibited nerve impulse production than in control neurones (Fig. 4). In the absence of morphine, the neurone was spontaneously active, and, when treated with naloxone, the dependent neurones fired impulses at high frequency.

Neuroblastoma × glioma hybrid cells

There is no doubt that a certain line of neuroblastoma × glioma hybrid cells (NG 108-15 cells), cultured in the presence of an opiate, develop a form of tolerance and dependence, expressed as an increased capacity to produce cyclic AMP[28,29]. Table 2 illustrates this form of dependence, in which challenge with naloxone in the presence of PGE$_1$ generated five times as much cyclic AMP in cells cultured for 48 h in the presence of morphine as in those cultured in its absence.

Although these cells possess opiate receptors[30], and the affinity of opiates for these receptors is correlated with their ability to inhibit adenylate cyclase[31], the extent to which induction of tolerance and dependence in NG 108-15 cells resembles that in normal neurones is uncertain, for three reasons. First, although NG 108-15 cells put out long processes, they are not arranged in a stable intercommunicating system, as are neurones of the CNS or myenteric plexus. Each NG 108-15 cell must be regarded as a distinct unit, functionally resembling its neighbours. Second, the NG 108-15 cells are of malignant origin and, unlike normal neurones, they reproduce themselves. Thus, in 10% calf serum in their usual culture medium, the cells double their number in about three days[32]. These cells, however, can develop their characteristic tolerance and dependence even when their reproduction is inhibited (W. A. Klee, personal communication). The third difficulty is to translate a change in level of cyclic AMP in the NG 108-15 cell into terms of excitation or inhibition of normal opiate-sensitive neurones. The different effects of adenosine illustrate this difficulty. In NG 108-15 cells, adenosine stimulates adenylate cyclase (Table 2) and thus acts acutely in the opposite direction to morphine[29]. In contrast, in individual opiate-sensitive neurones of the corpus striatum of rat brain *in situ*, both adenosine and morphine, administered by microiontophoresis, inhibit nerve impulse production[33]. Moreover, in glial cells cultured from the brain of perinatal mice, adenosine binds with two distinct receptors[34]. One of these inhibits adenylate cyclase and lowers the level of cyclic AMP, the other has the opposite effect.

It is tempting to suppose that, in the opiate-sensitive neurones of the rat striatum, the receptor activated by adenosine is one that inhibits adenylate cyclase. If so, the observed inhibition by adenosine of nerve impulse production in the striatal neurone might express a decrease in cyclic AMP; and hence a rise in cyclic AMP in the NG 108-15 cell might be translated as excitation in the opiate-sensitive neurone. This interpretation would be consistent with the specific and stereospecific inhibition of adenylate cyclase exerted by opiates and opioid peptides in homogenates or slices of brain *in vitro*[35–42], although their potency against adenylate cyclase and the potency of naloxone in antagonising their action are usually lower than might be expected from the affinities for opiate receptor in brain homogenates[43–45]. Attempts have so far failed to show, however, that opiates acutely inhibit nerve impulse production in individual neurones of guinea pig myenteric plexus[46] or of cat spinal cord[47] *in situ* through an inhibition of adenylate cyclase.

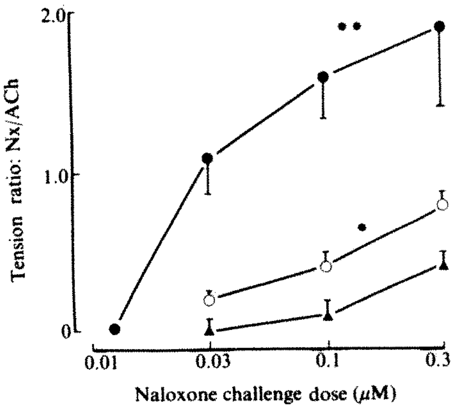

Fig. 2 Responses to naloxone challenge of segments of guinea pig ileum incubated for 24 h at 22 ± 2 °C in Krebs solution containing 70 μM hexamethonium (▲) or in this incubation medium, with the addition of 0.01 μM normorphine (○) or of 1.0 μM normorphine (●) (ref. 26). Tissues were set up for test at 37 °C in medium equivalent to that used for incubation and challenged 30 min later with naloxone (Nx). Tension ratio is the ratio of the maximal tension of contracture elicited by the challenge dose of Nx to that elicited bs a reference dose of 0.01 μM acetylcholine (ACh). *$P<0.01$, **$P<0.001$ for significance of difference of dose-response curve between normorphine-treated and control preparations.

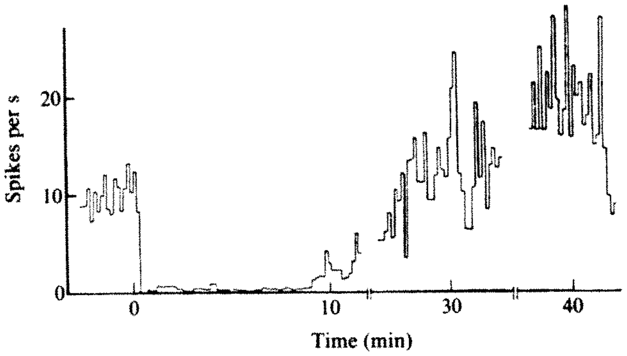

Fig. 3 Effect of prolonged application of morphine on frequency of nerve impulse production by a neurone *in situ* in the sensorimotor cortex of an opiate-naive rat[27]. Morphine was applied at 50 nA for 40 min from time zero. Breaks in the record are indicated by double vertical lines in the abscissa. For about 10 min, morphine suppressed nerve impulse production; thereafter production rose until, for the last part of the exposure to morphine, the neurone was producing impulses faster than it did before morphine.

Effects outside the neurone

The foregoing evidence shows that opiate dependence and associated tolerance can occur within the opiate-sensitive neurone; but it does not unequivocally indicate whether this neurone is their only site. That, as dependence deepens, preparations both *in vivo*[16,17,19] and *in vitro*[26] become responsive to very low concentrations of naloxone suggests that the opiate-sensitive neurone is also the primary and essential site of dependence.

There is, however, evidence that changes in other neurones may reinforce the effect in opiate-sensitive neurones. Thus, continued treatment of rats with morphine induces a supersensitivity to stimulation of adenylate cyclase by catecholamines in a cortical neurone downstream of that inhibited by opiate[48]. This supersensitivity is associated with an increase in postsynaptic receptors.

The opiate-sensitive neurone in a nervous system is necessarily subject to many influences from other neurones. Some of these could modify the induction or expression of dependence within the opiate-sensitive neurone. Such factors would include a diminished supply of endogenous opioid[49] or increased production of an endogenous opiate antagonist[50,51]. Since dependence can be induced, however, in the isolated ileum, these factors may be restricted in origin to cells in the neighbourhood of the opiate-sensitive neurone, or to the neurone itself.

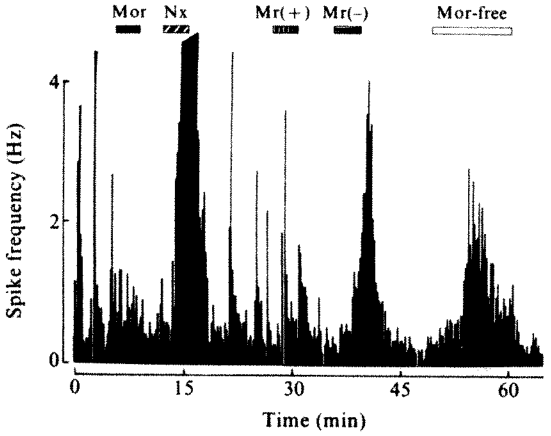

Fig. 4 Effect on a myenteric neurone of prolonged exposure to morphine in conditions of synaptic transmission blockade[23]. Before beginning the recording (time zero), this neurone had been exposed for 24 h to a solution containing morphine (1 μM), hyoscine (1 μM) and hexamethonium (300 μM). This solution continued to perfuse the tissue throughout the period of recording except for the time indicated by the open bar, when it contained only hyoscine and hexamethonium. During the periods indicated by the other bars, the perfusing solution contained additionally morphine (to a final concentration of 10 μM, Mor), naloxone (100 nM, Nx), Mr2267 (100 nM, Mr(+)) or Mr2266 (100 nM, Mr(−)). Note that the neurone was tolerant in that a very high concentration of morphine no longer depressed its firing. Naloxone caused a pronounced increase in firing (this reached 8–10 Hz), Mr2267 had no clear effect on the firing rate, but its active, (−) enantiomer, Mr2266 caused a marked excitation. This neurone was also excited simply by removing the morphine from the perfusing solution (Mor-free). In this and other experiments, second and subsequent exposures to antagonists did not cause as marked an excitation as did the first application.

Molecular mechanism of dependence

In the dependent neurone, the balance between excitation and inhibition has moved in favour of excitation. This is expressed both in an increased responsiveness to excitatory[52–54] and a decreased responsiveness to inhibitory[55,56] messenger substances. This change in balance seems to express an increased state of intrinsic excitation, shown by the high rate at which the dependent neurone fires nerve impulses either spontaneously or when challenged with naloxone (Figs 3 and 4). In the presence of such excitation, the growth of tolerance to endogenous opioid[49], possibly through induction of an enkephalinase[57], would be expressed as dependence.

This change in the balance of excitation may correspond with 'neuronal kindling' (ref. 25) or with 'supersensitivity' and 'subsensitivity' (refs 58, 59); but such terms do not provide a molecular mechanism for dependence. The principle that dependence occurs within the opiate-sensitive neurone enables us to discard several hypotheses of this mechanism and to retain for further consideration those concerning three aspects of neuronal function; (1) receptors for humoral messengers, whether opioid or non-opioid; (2) release of transmitter from the neurone terminal; and (3) second messenger systems connecting receptor binding and transmitter release. Although hypotheses, based on changes in receptors[4,25,59,60] or transmitter release[61,62], have been advanced, there is little supporting evidence. There is, however, strong evidence for a change in a second messenger system as the molecular mechanism of dependence. In theory, inhibition by opiate or other inhibitory substance of a process in a second messenger system could lead to tolerance and dependence, through a compensating hypertrophy in the system. This was first proposed for the cyclic AMP system[35], because opiates specifically and stereospecifically inhibited the E prostaglandin-stimulated adenylate cyclase of rat brain homogenate.

As we have seen, a compensating hypertrophy of cyclic AMP production in response to inhibition by opiate of adenylate cyclase has been clearly demonstrated in NG 108-15 cells[29]. There is evidence of four types that such hypertrophy also occurs in neurones of dependent animals. First, brain cyclic AMP production is raised in opiate withdrawal[63–68]. Second, injection of cyclic AMP into a cerebral ventricle of the dependent rat intensifies withdrawal jumping after naloxone challenge[69]. Third, withdrawal is accompanied by an increase in protein kinase activity in brain homogenates[68,70]. Lastly, a 'quasi-morphine withdrawal syndrome' indistinguishable in appearance from a true morphine withdrawal syndrome, can be elicited in opiate-naive rats by treatment with a potent phosphodiesterase inhibitor, such as 3-isobutyl-1-methylxanthine (IBMX), together with a low dose of naloxone[71,72]. The potency of phosphodiesterase inhibitors in eliciting this syndrome correlates with their potency as inhibitors of cyclic AMP phosphodiesterase in rat brain homogenate[73]. On the present evidence, therefore, an hypertrophy of the cyclic AMP system within the opiate-sensitive neurone in response to inhibition of adenylate cyclase emerges as the most likely explanation of opiate dependence.

Difficulties

Four seeming difficulties emerge from the above analysis. First, the extent to which sensitivity to morphine declines in the induction of tolerance and dependence is usually less than that to which sensitivity to naloxone increases[16]. Second, although naloxone is a potent antagonist of the acute actions of opiates[1], it rather weakly inhibits the induction of opiate dependence[18] or the inhibition by opiate of adenylate cyclase[35–42]. Third, in rat brain preparations *in vitro*, the potency of morphine as a specific inhibitor of adenylate cyclase[35–42] is usually less than might be expected from its affinity for the opiate receptor[43–45], although this does not apply to NG 108-15 cells[31]. Fourth, in individual neurones of the myenteric plexus or of the cat spinal cord, administration of neither cyclic AMP nor IBMX antagonised the acute inhibition by opiate of nerve impulse production[46,47], despite the evidence that opiates specifically inhibit adenylate cyclase in brain preparations *in vitro*[35–42].

These difficulties may disappear with continued experimentation. For example, although IBMX did not antagonise the acute inhibition by opiate of nerve impulse production in individual neurones of the myenteric plexus[46] or spinal cord[47], another methylxanthine, aminophylline, was recently found to do so in neurones of rat striatum[33]. If, however, the difficulties persist and sharpen, a second postulate may be needed. One possibility would be that a special variety of opiate receptor subserves dependence induction. Another would be that binding of opiate molecule with its receptor activates two distinct processes: one, the inhibition of adenylate cyclase that leads to dependence; the other, so far unidentified, leading to the acute agonist action. As usual, new insights raise new problems and require new experiments.

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ARTICLES

Climate and sea level in isotope stage 5: an East Antarctic ice surge at ∼95,000 BP?

John T. Hollin

Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309

Six high-resolution records correlated with marine isotope stage 5 suggest that substage 5c was essentially interglacial, and was terminated by a catastrophic cooling. Over sixty ^{230}Th dates indicate that the sea level in substage 5c rose to at least −2 m. Amino acid ratios, archaeology, pollen and lithostratigraphy suggest that the sea later jumped to about +16 m. The combination of the cooling and the large jump points to an East Antarctic ice surge, at ∼95 kyr BP.

BUDD AND MCINNES[1] suggest that "periodic surging of the large ice sheets may have been the rule rather than the exception". In Antarctica, Budd and McInnes envisage minor surges every 5 kyr or so, and major surges, such as those considered by Wilson[2], Hollin[3] and Flohn[4], every 100 kyr or so. I have pointed out that surging in Antarctica would have caused distinctive jumps of sea level: large, fast (a few hundred years or very much less?), brief (a few thousand years?), cool and, in the special case of Wilson's suggestion that surges caused the Pleistocene ice ages, late in each interglacial. I reviewed some lithostratigraphic and biostratigraphic evidence for such a jump late in the last interglacial, from below 0 m to about +16 m in stable areas. In 1972, impressed with the cooling events in Fig. 1*b*, *c*, *d*, and with the Mediterranean and Red Sea dates below, I estimated 95 kyr BP as the most likely date for this jump[5]. Later facing the evidence that the last interglacial *s.s.* was represented by marine oxygen isotope substage 5e and dated from about 125 kyr BP (ref. 6), I thought that if the jump was real it must date from roughly 120 or 115 kyr BP (ref. 7). I review here new evidence that the sea rose almost certainly to −2 m and probably to 16 m at about 95 kyr BP. Biostratigraphic evidence that the rise was late in 'the last interglacial' is explained by evidence that not only substage 5e but also 5c was essentially interglacial in character.

Climate

Figure 1*a* is an equatorial record of marine oxygen isotope stage 5. The global record of stage 5, based on nearly 100 cores, forms our best framework now for thinking about the last interglacial,

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s.l.[8]. However, in the marine isotope record, substage 5c usually appears more 'interstadial' than 'interglacial'; possible reasons are given later. Figure 1*b, c, d* show the Gulf of Mexico[9], Orgnac (France)[10] and Camp Century (Greenland)[11] rapid cooling events, whose interpretation as the result of an Antarctic ice surge has already been discussed[5]. Figure 1*e, f, g* come from high resolution pollen diagrams which appear to cover stage 5. Figure 1*e* is from the Tenagi-Philippon, Greece[12,13], where the oak forest of Wijmstra's zone S 'Drama', reflecting temperatures "a little lower than they are today", changed to cold steppe, reflecting "an average summer temperature at least 5–8 °C lower than today" (ref. 14) in one counting level, here estimated at <1,000 yr. Figure 1*f* is from Vandalia, Illinois[15]. If Grüger is correct in assigning his zone 1 to the 'Illinoian', that is, isotope stage 6, then the possible forest to prairie 'catastrophe' at the end of his zone 2c correlates very well with the coolings above. Figure 1*g* is from Woillard's[16] profile from Grand Pile, France, in which she recognises three distinct interglacials in stage 5 time. Her St Germain I interglacial correlates with substage 5c, but in this case its exact position on the horizontal axis was uncertain. The deforestation at 1,380 cm seemed much higher in the profile than the initial deterioration in the detailed diagram of the vegetation. In Fig. 1, I considered the most important climatic break was at about 1,460 cm (where the still high *Carpinus* pollen would match that in the massive brickearth at West Thurrock, UK below[7]). Now Woillard has reported[17] that there was indeed an abrupt vegetational and climatic break at 1,455 cm.

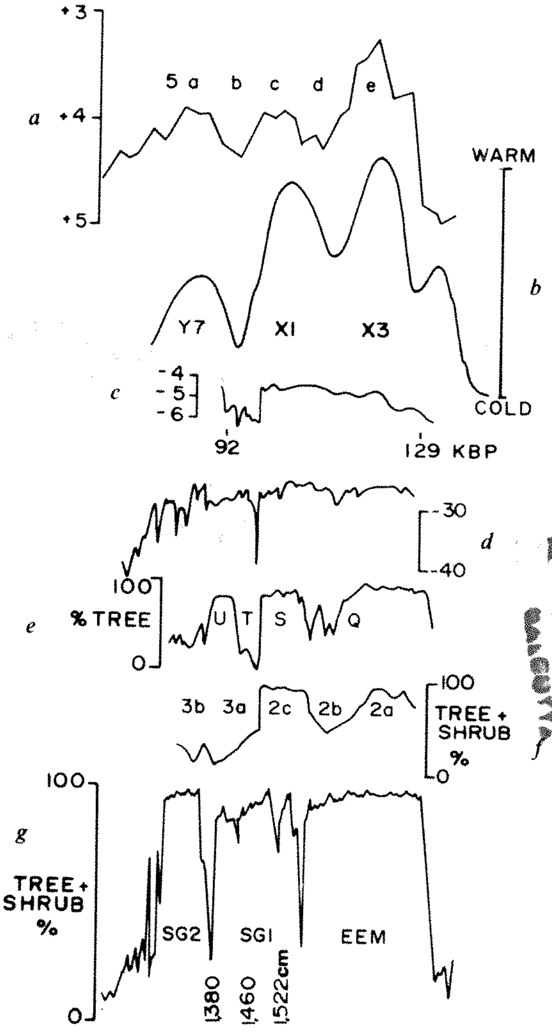

Fig. 1 Correlations with marine oxygen isotope stage 5. *a*, Isotope substages 5a–e in core V19-29 from east equatorial Pacific[8]. Scale on left is isotope composition of benthic foraminifera in ‰. *b*, Biological climatic curve from western Gulf of Mexico[9]. Y7 and so on are foram zones. *c*, Isotopic curve from Orgnac stalagmite, France[10] in ‰. Numbers below are ^{230}Th age determinations. *d*, Isotopic profile from Camp Century ice core, Greenland[11] in ‰. *e*, Pollen diagram from the Tenagi-Philippon, Greece[12,13]. U and so on are pollen zones. *f*, Pollen diagram from Vandalia, Illinois[15]. 3b and so on are pollen zones. g, Pollen diagram from Grand Pile, France[16]. SG2 and so on are Woillard's St Germain II and I and Eemian interglacials. Numbers below are depths in cm.

Two important points emerge from Fig. 1. First, except in Fig. 1*a*, it seems that the substage 5c climate was essentially interglacial at the localities discussed. This shows clearly in the physical evidence from Orgnac and Camp Century, is implied by Grüger for his zone 2c and is argued specifically by Woillard[18] for her St Germain I. Two North American speleothem records suggest that substage 5c was actually warmer than 5e (ref. 19). Proglacial meltwater lakes in Antarctica were particularly widespread in 5c time (ref. 20, p. 182, Fig. 4). I avoid defining 'interglacial', and do not think that substage 5c was as warm as 5e in every part of the globe, but in view of the evidence above it is not surprising that the sea in substage 5c rose slowly to within 2 m or so of its present level. Second, in all six of the high resolution records, it seems that substage 5c was terminated by a 'catastrophic' cooling. At Camp Century, where the time resolution is best, the cooling may have occurred in <100 yr.

Sea level

That the Gulf of Mexico cooling precedes a prominent ash deposit there, makes an Antarctic surge rather than a volcanic outbreak the most probable cause of these possible coolings[5,21]. In any case, this particular cause is subject to the sea-level test. Traditionally (see, for example, refs 22–26) two sea levels—at 6–8 and 15–20 m—are assigned to the last interglacial in stable areas away from, for example, plate boundaries and the Pleistocene ice sheets. In the past 15 yr over sixty ^{230}Th/^{234}U dates have been obtained from geomorphological features, chiefly reefs and notches, at the 6–8 m level, notably in the Pacific and Indian oceans[27] and in detailed studies in Aldabra[28], Oahu[29] and the Bahamas[30]. The mean of these dates is 125 kyr BP, so that the 6–8 m level seems to be from early in the last interglacial. The 15–20 m level is less pronounced geomorphologically, and has been neglected in dating studies: does it really belong to stage 5, or is it from an earlier interglacial? On the basis of its associations with Mousterian archaeology and cool molluscs, many early authors attributed it to the end of the last interglacial and the beginning of the last glaciation (ref. 31, pages 1256, 1271). In 1965 I suggested that such a high late level might be explained glacio–eustatically by an Antarctic ice surge[3]. I now consider the evidence for this hypothesis which leads to the sea-level scheme of Fig. 2*a*.

The classical area for the study of Pleistocene sea levels has been the western Mediterranean and Morocco. Work beginning in the nineteenth century was reviewed by Osborn and Reeds[32], who called the correlation of a 20–18 m shoreline, a Mousterian archaeology and the early Würm glaciation "the most firmly established of any". In this area, Stearns and Thurber[33,34] conducted a pioneering ^{230}Th dating campaign on molluscs. Ten of their results fell between 140 and 115 kyr BP and 10 between 95 and 75 kyr BP. Of their dates for which elevations are given, the mean for the three shells in the older group is 6.5 m, and for the five in the younger is 11 m. Even if their young sample L934E from 33 m is omitted, the mean for the remaining four is still 5 m. Young sample L787D from 10 m comes from a conglomeratic apron reaching above 18 m. Obviously, these results favour early schemes and the present paper. However, there are two criticisms.

First, Kaufman *et al.*[35] concluded that the ^{230}Th method on individual molluscs was giving correct ages no more than half the time. But, as the 140–115 kyr BP group fits very well the 125 kyr BP mean of so many coral dates, it seems likely that the 95–75 kyr BP group also corresponds to some real event, and concentrations of dates in this range continue to be obtained from the Mediterranean[36]. Especially important are the results of Bernat *et al.*[37] from Spain, where seven *Strombus* molluscs yielded a mean age of 97 kyr BP, with a standard deviation of only 4,900 yr. Sanlaville[38] describes a double transgression to 10 m in the Lebanon, associated with two mollusc dates of 90 kyr BP, and with archaeology from *le premier interstade würmien*. Second, the Mediterranean is a plate boundary area, and Hey[39], for example, has stressed the impersistence of many of its raised shorelines. However, Gigout *et al.*[40,41] claim that in the area of Bernat's dates, the lowest raised strandline can be traced for 400 of the 1,100 km they studied. We need more work in stable areas but, to test the surge hypothesis, it should be sufficient to see if the 95 kyr BP transgression was relatively higher than the 125 kyr BP.

Table 1 Summary list of ^{230}Th/^{234}U dates suggesting a substage 5c sea level at −2 m or above

Area	No.*	Dates (kyr BP)	Sea level (m)	Refs
Western Mediterranean, Morocco	10 M	95 to 75	18?	33, 34
Algeria	3 M	102, 93, 90		36
Spain	7 M	97 ± 4.9		37
Lebanon	2 M	90, 90	⩾10	38
Black Sea	3 M	88 to 66	10 to 15	52
Egypt	4 C	92, 89, 89, 70	⩾8	53
Madagascar	2 C	94, 85	⩾1	54
Australia	8 M	101 ± 14		57
South-west Pacific	6 C	95 ± 4	⩾−2	61
Virginia area	11 S	84 to 59	14?	65
Florida	1 C	95 ± 9		68
Bahamas	4 C	112 to 94		30
Bermuda	1 C	97 ± 9	>0	71
Canary Islands†	M	100	8?, 16?	86
Sudan	1 M	91	⩾9, 17?	87
Mangaia	2 C	110, 90	>2	27
Bonaire		103 (C) 93 (M)		88

* M, molluscs; C, corals; S, solitary corals.

† Last four areas are not discussed in the text, but merit study.

In the UK, I have summarised[7] much evidence for a double sea level in the last, 'Ipswichian' interglacial: an early, 7-m level represented in the Thames estuary by brackish plants at Ilford, an inferred floodplain surface at Aveley, and tidally laminated beds at Crayford; and a late, 16-m level represented by a polleniferous aggradation at Aveley and West Thurrock, by Hodgson's sand and gravel sheet in Sussex, continuous from 0 to 14 m, and by the raised beach at Portland, rising to 20 m, and with modern but cool molluscs. Kidson[42] suggests a double last interglacial sea level in Somerset, at 6–9.5 then 18–20 m, the later higher level represented by the Burtle Beds. Bowen[43] has long argued for a double level in Wales.

Recently, Miller *et al.*[44] reported an amino acid, relative dating campaign in the Thames estuary: modern D-alloisoleucine: L-isoleucine ratios (total, = free plus peptide-bound) are close to zero. Ratios from the freshwater mollusc *Corbicula fluminalis*, from Ilford, Aveley and Crayford above, fell into a well defined group with a mean of 0.21. Andrews *et al.*[45] reported on a similar study on coastal exposures. Ratios from the marine mollusc *Patella vulgata*, from Portland and the Burtle Beds, had a mean of about 0.12. The marine *Macoma balthica* from the Burtle Beds had a mean of 0.14. The small *Patella–Macoma* difference may be a species effect. But the large difference between *Corbicula* and these shells suggests that the latter may indeed be appreciably younger, especially as at the one locality where it is possible to compare the freshwater and marine ratios (Selsey–Selsea Bill; ref. 44, Tables 1, 2), four *Corbicula* all have ratios less than one *Macoma*.

An early 7-m level in the UK would correlate very well with the global 125 kyr BP, substage 5e level; is there any evidence that a late 17-m level could be from a substage 5c interglacial? Sutcliffe has argued that the UK Ipswichian was at least twofold (see ref. 46, pages 223–224 for references and discussion). He claims that the mammals at Trafalgar Square, in particular *Hippopotamus amphibius*, show it to be from a younger interglacial than Ilford, Aveley and Crayford above. I have recently realised that *Corbicula* is missing from just those two Thames sites, Trafalgar Square and West Thurrock[7], with *Hippopotamus*. In fact, a literature check has revealed no UK site where these two fluvial animals undoubtedly lived together. For example, there is no overlap between the seven sites in Sparks's[47] review of the last interglacial *Corbicula* and the four in Stuart's[46] review of last interglacial *Hippopotamus*. A third biological argument for multiplying the Ipswichian is provided by the contrast between the beetles from Aveley and the more exotic ones at Trafalgar Square (and Bobbitshole, Ipswich)[48]. Woillard's[16] diagram from Grand Pile suggests that it may be difficult to distinguish 5e and 5c interglacials by the pollen method, but Ilford and Aveley, Trafalgar Square and West Thurrock, plus the coastal sections should clearly be the subject of a further amino acid study.

A difficulty with this multiplication is that, if the *Corbicula* from Ilford, Aveley, Crayford (and Stutton[7,44]) are from substage 5e, how can the massive brickearth at these localities[7] be attributed to a surge in substage 5c? The recent pollen work of Woillard[17], plus the sea-level observations of Chappell and Thom[49], raise the possiblity of another (minor?) surge at the end of 5e also (Fig. 2*a*). (Rough calculations of the ensuing ice build-up rate in Antarctica (ref. 7, p 49) and perhaps the north suggest that a surge high sea stand probably lasted longer than Chappell and Thom assumed). If the resulting transgression rose to <16 m, its littoral deposits would have been largely destroyed by the apparently major, 5c transgression that is the main concern of this article.

In northern France, Lower Normannian (up to 5 m) and Upper Normannian (12–18 m) beaches have traditionally been assigned to the last interglacial. Guilcher[50] writes "it may be that the Lower Normannian is older in some areas". In northern Europe, Charlesworth (ref. 31, p 959) reviews a large amount of evidence for a double sea level in the last interglacial. The second, 'Portlandia Sea', 'White Sea' transgression contains the *Carpinus* typical of the second halves of the Eemian and St Germain I interglacials. Van der Heide[51] writes of the White Sea area "two transgressions are known. The first has a molluscan

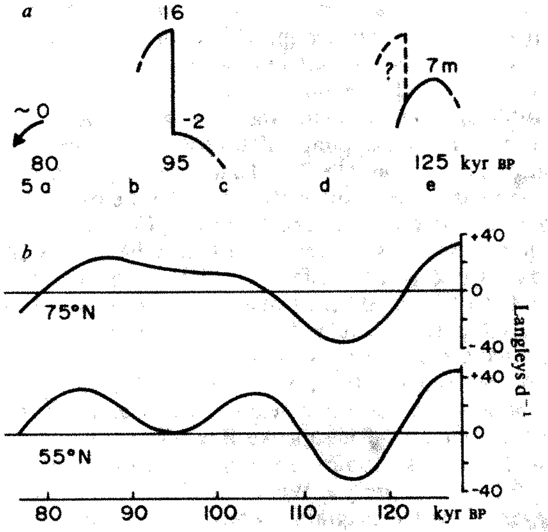

Fig. 2 *a*, Sea-level scheme for isotope stage 5, showing suggested major surge at about 95 kyr BP and possible minor surge at about 120 kyr BP. Figures above curves are elevations in m. 5a–e are correlations with isotopic substages. *b*, Deviations of solar radiation from their 1950 AD values, for the caloric Northern Hemisphere summer, for 75° N and 55° N, plotted from ref. 85, Table B.

fauna including somewhat warmer elements. The fauna of the second is markedly colder. Several authors believe that a connection between the White Sea and the Baltic Sea originated during this transgression". In the Black Sea, on the Caucasus coast, Muratov *et al.* (ref. 52, Table 1) assign to the Riss-Würm, Mikulino interglacial a double 'Karangat' transgression, to 5 then 10–15 m, associated with mollusc dates of 88–66 kyr BP.

In the Red Sea, Veeh and Giegengack[53] review several mollusc dates in the 90 kyr BP range, and report their own dates of 92, 89, 89 and 70 kyr BP from corals up to 8 m in Egypt. Battistini *et al.*[54] report coral dates of 94 kyr BP at 0 m and 85 kyr BP at 1 m from the area of Madagascar. From South Africa, near Port Elizabeth, Davies[55] reports that "Amino acid ... ratios in molluscs ... indicate that a transgression to +15 m occurred after a sea level at +8–9 m that is presumed to be Eemian in age on a variety of evidence. The transgression may be ... from a surge of the ... Antarctic ice sheet."

In Australia, in New South Wales, coral dates between 143 and 112 kyr BP are assigned to a sea stand at 5 ± 1 m (ref. 56). But in Victoria, Schornick[57] has reported eight mollusc dates with a mean of only 101 ± 14 kyr BP. Outside New South Wales, the sea level in the last interglacial is thought to have been much higher; 21 m in Victoria[58] and 21 m by many workers in Bass Strait and Tasmania[59]. As in Sussex above, the deposits involved seem to be unbroken down to below 0 m. That this level is slightly higher in Australia than elsewhere is what we might expect if the source of the water involved was (West or East) Antarctica (ref. 60, Fig. 4).

Table 2 Summary of data suggesting a stage 5 sea level at ~16 m

Area	Principal evidence	Refs
Western Mediterranean	Archaeology, ^{230}Th dates	32–34
UK	Lithostratigraphy, pollen, amino acid ratios	7, 42, 44, 45
Black Sea	^{230}Th dates	52
South Africa	Amino acid ratios	55
Australia	Lithostratigraphy	58, 59
Virginia	Lithostratigraphy, amino acid ratios	65, 67
Bermuda	Lithostratigraphy	70

In the Pacific, Bernat *et al.*[61] report from New Caledonia and the Loyalty Islands four coral dates with a mean of 122 ± 3 kyr BP and six with a mean of 95 ± 4 kyr BP. Most of their samples were recrystallised, but the two with 100% aragonite came from the younger group. This area is close to a plate boundary, and the only island on which it is possible to compare elevations is Lifou. There, three of the older dates seem to come from within an old reef or cliff (the substage 5e one?), raised to about 11 m. One of the younger dates comes from the surface of the platform below this, at ~2 m. To test the surge hypothesis, it would be good to date more surface samples, below and especially above 11 m. In Japan, Kikuchi (ref. 62, Fig. 11) and Naruse have deduced double sea levels above 0 m in the last interglacial. Machida[63], working with fission track dates, estimated absolute sea levels at 100 and 80 kyr BP at about −9 to −3 m. Hopkins[64] deduced a double sea level in the last interglacial in Alaska, but thought that the early level was higher.

Marine deposits on the east coast of the US have been the subject of an extensive amino acid study by Belknap[65]; his Fig. 44, on the Delmarva–Chesapeake Bay region, shows concentrations of dates at about 125 and 95 kyr BP (although the intervening unconformity at Wailes Bluff does not fit easily into Thompson's[66] pollen diagram). Many of these dates are from coastal exposures, and it seems likely that most of the shells from the thin, landward edges of the marine formations have been leached away. However, intensive lithostratigraphic work on the extent of these formations in Virginia was carried out by Oaks and Coch[67]. They assigned to the last interglacial a Great Bridge Formation, related to a sea stand at about −2 m, which was followed by an unusually rapid transgression to a Norfolk Formation level at 14 m. The sea then regressed below 0 m, leaving the deposits of the Kempsville, Londonbridge and Sand Bridge Formations. Five amino acid dates between 100 and 91 kyr BP seem to come from the Norfolk Formation. On the other hand, 11 solitary corals from the Norfolk and Kempsville Formations have a mean ^{230}Th age of only 68 ± 9 kyr BP (ref. 65, appendix G). Whether this discrepancy has a geological or a chemical origin remains to be discovered. In Florida, coral dates include one of 95 ± 9 kyr BP from the Key Largo Limestone at Windley Key[68].

In the Bahamas (ref, 30, Table 2), perhaps the coral conglomerates dated to 103, 105, 94 add 112 kyr BP are reworked from a primary substage 5c sea stand. In Bermuda, Land *et al.*[69] recognised a sea-level maximum of 5 m at 120 kyr BP (four dates), then a regression to below 0 m, then a transgression to between 11 and 20 m, dated, however, to 130 kyr BP. Vacher[70] studied this second transgression more closely; he concluded that it was preceded by a sea level "slightly below present datum", that it reached 15 m and, from lithostratigraphic evidence, that it was "abrupt and rapid, and may signify a catastrophic surge in Antarctica". Harmon *et al.*[71] confirmed the 120 kyr BP dating (mean of seven dates 124 ± 6 kyr BP), deduced a regression to −8 m or below then a second transgression at −6 m at about 100 kyr BP, redated the 130 kyr BP coral four times, and obtained a mean age of 97 ± 9 kyr BP for it. All these results fit Fig. 2*a* well. However, whereas the evidence for the 15-m sea level comes from seaward dipping calcarenites on the Bermuda north shore, the 97-kyr BP coral came from the south shore, from a deposit which Harmon *et al.* interpret merely as a storm beach, thrown up from a sea stand at about 0 m. Against any higher rise is the absence of any aragonitic, marine overgrowth on their speleothem 73018, from about 7 m and dating from 162 kyr BP. But perhaps this speleothem was protected against a brief transgression by the freshwater table. We need now some dates from the north shore calcarenites. However, in the surge hypothesis, these beds may represent, not conventional marine deposits, but rather the rapidly reworked, semi-consolidated terrestrial deposits and fossils from the 125–95 kyr BP regression: like the West Thurrock brickearth, a possible diluvium[7].

Discussion

The ^{230}Th dates above (Table 1) strongly suggest that sea level in substage 5c rose to at least −2 m. Many of the mollusc dates, plus the amino acid ratios, archaeology, pollen and lithostratigraphy (Table 2) suggest that the sea later rose to ~16 m. The data from Sussex, Portland and the White Sea suggest that the later rise was cool. Thames, Virginia and Bermuda authors have all argued that it was rapid. Unusually rapid rises to 15 m have been argued for Guernsey[72] and Cyrenaica (ref. 73, p 54), although here no dating is available. Obviously, these data support the surge hypothesis. However, they also raise several questions.

(1) An immediate worry with Fig. 2 is the great size of the sea level jump: 18 m. The present distribution of the world's ice, in terms of sea-level equivalents, is roughly: East Antarctic ice sheet 60 m, West Antarctic ice sheet 6 m, Greenland ice sheet 6 m, lesser ice caps and valley glaciers 0.3 m. If the jump is real, then it requires a surge, or ungrounding (ref. 74, p 185), of the world's thickest ice, most probably from Wilkes Land, less probably from Queen Maud Land (see ref. 75 for bedrock and ice thickness maps).

(2) Veeh[27] puzzled why little trace of Stearns and Thurber's younger dates appeared in his coral study. We see now that the primary 5c reef, at ~−2 m, is out of sight today in stable areas. And the secondary, surge transgression would have been too rapid for reef growth, and would be marked chiefly by a sheet or patches of sand, with relatively cool molluscs. However, as the water cleared, and the initial cooling waned, some coral growth would have recommenced in low latitudes, and this is perhaps what we see in the Red Sea, Madagascar, and so on.

(3) Many workers remark that their elevations do not fit 'the Barbados model' (ref. 76), of 122, 103 and 82 kyr BP sea stands at +6, −13 and −13 m respectively. But Stearns[77] re-evaluated the Barbados (and New Guinea[78]) elevation data, and concluded that the 103 and 82 kyr BP stands were in fact close to 0 m. The present data strongly support him. In a third uplifted area, in Atauro (ref. 79, Fig. 9), if reef terrace 1b in fact continued developing until 95 kyr BP it would reflect an absolute elevation not far below the −2 m given here.

(4) The Barbados model gained support from an early foraminiferal oxygen isotope study[80], which suggested a substage 5c stand at about −13 m. Subsequently, Barbados mollusc isotope data have suggested a 5c stand at −28 m, and Barbados coral isotope data a 5c stand at −43 m (ref. 81). Perhaps these disagreements can be resolved by interpreting the isotopic data more in terms of temperature than ice volume. The mean isotopic change, from substage 5e to 5c, involved in the results above was about +0.4‰. In the present scheme, the sea dropped from +7 to only −2 m in this time, so we can attribute only +0.1‰ of the change to an ice volume effect[8]. The remaining +0.3‰ may represent a small cooling, which we may calculated[82] as 1.3 °C. Such a cooling in this area is indeed suggested by Kennett and Huddlestun's planktonic biological data, in Fig. 1*b*.

(5) An even lower substage 5c stand is suggested by the 5e to 5c change of +0.7‰ in the benthic isotopes of Fig. 1*a*. This disagreement between stage 5 sea levels and the benthic isotope data has already been discussed, by Broecker[83]. Again, he suggests a cooling, by about 2 °C, late in substage 5e. He draws attention to a possible analogous benthic change of over +0.2‰ late in the Holocene stage 1, a change which obviously cannot be interpreted in terms of ice volume.

(6) The emphasis here has been on finding out if Antarctic surges do in fact happen, and it may be premature to discuss their possible role in the spectrum of climatic change[2,84]. However, one climatic point may be made. Not only a catastrophic cooling at the end of 5c, but also the whole of the subsequent cold substage 5b is hard to explain by insolation changes. During 5b time summer insolation north of 55° N was greater than today's, and north of 75° N was continually increasing (Fig. 2*b*). Substage 5b seems to be an ice age that is better explained by a surge than by insolation changes. Hitherto, cold substage 5d may have seemed a case of the opposite (Fig. 2), but now, in the light of refs 1, 17 and 49, and of the possibility of a minor surge at ~120 kyr BP, this no longer seems certain.

The sea-level scheme of Fig. 2 incorporates a very large quantity of data. We now need more quality. Above all, we need ^{230}Th coral dates up towards 16 m in stable areas, although care must be taken to distinguish the possibly thin and patchy deposits of a surge from the older deposits and geomorphology beneath. It is just possible that the uppermost littoral deposits at 16 m are the results of the minor, substage 5e (120 kyr BP) surge suggested above, but most of the present evidence favours their attribution to a major, 5c, 95 kyr BP surge. If the surge hypothesis is wrong, its disproof could come deposits at about 10 m that remained continuously freshwater after 125 kyr BP (see refs 3 and 5, and Bermuda speleothem 73018 above).

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Enriched mantle: Nd and Sr isotopes in diopsides from kimberlite nodules

Martin Menzies & V. Rama Murthy

Department of Geology and Geophysics, 108 Pillsbury Hall, University of Minnesota, Minneapolis, Minnesota 55455

Nd and Sr isotope compositions of diopsides from micaceous garnet lherzolite nodules found in kimberlite pipes at Bultfontein and Kimberley, South Africa are reported. The diopside data extend the mantle array, as defined by uncontaminated oceanic and continental basalts, to radiogenic $^{87}Sr/^{86}Sr$ *values. These data are used to demonstrate the ancient enriched, grossly heterogeneous nature of the sub-continental mantle, which complements the predominantly depleted nature of the sub-oceanic mantle.*

THE chemical character of the sub-continental mantle is the subject of considerable speculation. Most of our knowledge comes from heat flow and seismic data[1,2] or analysis of mantle-derived materials. Thermal and seismic studies point to a continental mantle more depleted in magmatophile elements than the sub-oceanic mantle[2]. Yet one of the principal conclusions from the interpretation of continental basalt data is that the continental lithosphere is essentially undepleted[3] in the light rare earth elements (REE). However, note that interpretation of the Sr and Nd isotope geochemistry of continental volcanic rocks is severely hampered by uncertainties concerning contamination by crustal material[4,5]. Thus considerable controversy has arisen over the extent of contamination and whether or not the range in isotopic composition reflects source heterogeneity[3] or variable degrees of crustal assimilation[4,5]. Recent interpretations of Rb–Sr isotope geochemistry assume that contamination effects are negligible[3] but combined Nd- and Sr-isotope studies have elegantly demonstrated 'selective' contamination of basalts with crustal granulites and amphibolites. To alleviate these problems one can study peridotite nodules believed to be of mantle origin. However, care must be taken as minor amounts of contamination undoubtedly occurred during or after transportation of the mantle fragments to the surface. For example, recent isotopic studies of alpine peridotites have shown profound alteration during and after obduction[6]. Similarly, alkali basalt nodules are occasionally contaminated by the host basalt and some kimberlite nodules may have been 'metasomatised' by the host kimberlite[7,8]. To eliminate any possibility of such contamination recent isotopic and trace elements studies[6,9] have concentrated on carefully separated, inclusion free, mineral separates.

Diopside separates from these garnet lherzolites were chosen for analysis here because of their favourable abundances of Nd (20–50 p.p.m.) and Sr (150–400 p.p.m.), and the low Sm/Nd ratios (0.05–0.10), as well as the low Rb/Sr ratios (Table 1).

The analytical procedures used in this laboratory for Nd and Sr isotopes have been described elsewhere[10,11]. Separation and purification procedures for the mineral fractions have also been outlined previously[6,12].

Nd and Sr isotopes in mantle diopsides

The Nd- and Sr-isotope compositions have been measured in diopsides for eight lherzolite nodules and the Nd isotopic composition in diopside from a ninth nodule (Table 1, Figs 1 and 2). The diopsides have a considerable range in $^{87}Sr/^{86}Sr = 0.70465–0.70752$ and a similarly large range in $^{143}Nd/^{144}Nd = 0.51257–0.51195$. The $^{87}Sr/^{86}Sr$ ratio of co-existing garnet was measured in one nodule and was found to be identical to the co-existing diopside (Table 1). Secondary micas found in two nodules have radiogenic $^{87}Sr/^{86}Sr$ ratios and low $^{143}Nd/^{144}Nd$ ratios (Table 1). The abundance of mica in these nodules can present a severe contamination problem due to the high Sr content (~500 p.p.m.). However, minute inclusions of mica in diopside would tend to produce a linear relationship between Sr content and $^{87}Sr/^{86}Sr$. This was not found to be the case and, furthermore, each diopside was carefully handpicked and inspected for adhering mica flakes or inclusions. The identical $^{87}Sr/^{86}Sr$ ratio in co-existing garnet and diopside confirm the purity of our separates. It seems highly unlikely that random contamination of diopside (Sr = 140–400 p.p.m.) and garnet (Sr = 0.2 p.p.m.) with secondary mica flakes would result in identical $^{87}Sr/^{86}Sr$ ratios. Contamination would tend to dramatically increase the Rb/Sr and $^{87}Sr/^{86}Sr$ ratio of the garnet and diopside.

The most interesting aspect of the Sr and Nd data for the diopsides is the linear anticorrelation of $^{143}Nd/^{144}Nd$ with $^{87}Sr/^{86}Sr$ (Fig. 1). The data extend the 'mantle array', that was originally observed in uncontaminated oceanic and continental basalts[13,14], to $^{87}Sr/^{86}Sr$ values of 0.7075. Clearly mantle-derived liquids can have high $^{87}Sr/^{86}Sr$ ratios[15] and low $^{143}Nd/^{144}Nd$ ratios. All of the diopsides represent mantle regions that have a time-integrated light REE enrichment (Sm/Nd ratio < the bulk earth value). Other available data on garnet lherzolite nodules[13,16,17] reveal a Nd- and Sr-isotopic composition coincident with that of oceanic basalts. These lherzolites represent mantle with a time-integrated light REE depletion (Sm/Nd ratio > the bulk earth value). Clearly kimberlite nodules represent random samples of an inhomogeneous mantle characterised by both a LREE enrichment and depletion. Similarly data from alkali basalt nodules (ref. 18 and M.A.M. unpublished data) indicate that spinel lherzolite nodules represent mantle regions with both a time integrated light REE depletion and enrichment. These data have been summarised in Fig. 2 and compared with available basalt data.

Preliminary analyses of micas from garnet lherzolite nodules, plot off the mantle array as defined by the diopside data. Petrographic studies[8] indicate a secondary origin for these micas, that may involve the host kimberlite. However, Nd isotopic studies[17] of several fresh kimberlites indicate a source in the mantle with a chondritic REE abundance. This contrasts markedly with the two kimberlites analysed by DePaolo[19]. One highly serpentinised sample has a depleted source region while a slightly serpentinised sample is derived from an enriched source region. As these kimberlites are variably serpentinised their Nd and Sr isotopic compositions may reflect secondary processes. At this stage the mica data are difficult to interpret but we can say that a simple connection with the kimberlite magma is not indicated.

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Sub-continental mantle

Ultramafic nodules found as spinel and garnet lherzolites in alkali basalts and kimberlites provide information on the isotopic and chemical constitution of the continental mantle. Mantle depletion and enrichment events will be recorded in these nodules throughout geological time. Rb–Sr[9,20] and Sm–Nd[16,21] measurements of minerals in some alkali basalt and kimberlite nodules record ages up to 3,000 Myr indicating that certain regions of the mantle have remained isolated for long periods of time. These ages may record ancient depletion events associated with continent formation, while Rb–Sr[22] and Sm–Nd (M.A.M., unpublished data) studies of metasomatised nodules may record recent enrichment events (for example, ≈200 Myr) associated with alkaline magmatism. The consistent and wide variation in the Sr isotopic ratio of diopsides in mantle nodules is believed to reflect this gross mantle heterogeneity[15,23]. The Sr and Nd data presented here support this contention and demonstrate that the sub-continental mantle contains regions with both time-integrated light REE enrichment and depletion (Figs 1 and 2).

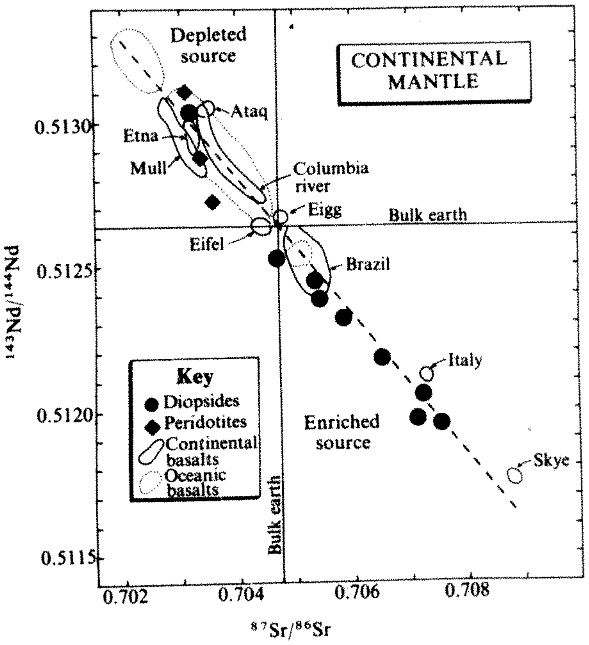

Fig. 1 ^{143}Nd/^{144}Nd and ^{87}Sr/^{86}Sr variation in diopsides from six kimberlite nodules (●) and one alkali basalt nodule from Nunivak, Alaska[24]. Comparative sources: Ataq[24], Mt Etna and Roccamonfina[4]; Mull, Eigg and Skye[5]; Eifel[27]; Columbia River[25]; and Brazilian flood basalts.[19]. Whole rock kimberlite nodule data[13] (◆) are shown for comparison.

Continental basalts are obviously derived from the sub-continental mantle and would be expected to reflect the gross heterogeneity described above. Figure 1 summarises available Nd and Sr isotopic data on continental basalts that are believed to represent uncontaminated mantle derived liquids. Basalts from Ataq, South Yemen[24]; Mt Etna, Italy[4]; Columbia River, US[25]; Rio Grande Rift[26] and other localities originated in depleted mantle regions similar to the source of oceanic basalts (Sm/Nd > the bulk earth value). However, continental flood basalts from Brazil[19] and rift valley basalts from Eifel, Germany[27] are derived from enriched (Sm/Nd < the bulk earth value) and chondritic (Sm/Nd ≈ the bulk earth value) source regions respectively. Nd and Sr data for uncontaminated basalts exhibit a considerable range in Nd and Sr isotopic composition that overlaps with that observed in diopsides from mantle nodules (Fig. 2). These data are therefore compatible with a grossly heterogeneous sub-continental mantle containing both depleted residual and enriched portions.

To our knowledge, very few suites of 'uncontaminated' basalts have been reported with low ^{143}Nd/^{144}Nd and high ^{87}Sr/^{86}Sr ratios. In most cases contamination with a crustal component has produced marked deviations[13,14] from the 'mantle array'. If we use the mantle array as a baseline against which contaminated suites can be compared, we may be able to define the approximate Nd and Sr isotopic composition of the 'mantle-derived liquid' that was subsequently contaminated during ascent through the continental crust. In the case of the Roccamonfina volcanic rocks from southern Italy[28] one sample (Fig. 1) has a Nd and Sr isotopic composition coincident with the mantle array as defined by the diopside data. Possibly this basalt represents a relatively uncontaminated volcanic rock derived from an enriched mantle. The associated volcanic rocks that plot to the right of the array represent mantle derived liquids variably contaminated with a crustal component[29], or uncontaminated liquids derived from metasomatised mantle[28]. One can also speculate that the Nd and Sr isotopic composition of an epigranite from Skye, Scotland[5,30] may approach that of the original mantle derived liquid. However, the chemically evolved nature of this rock indicates that it is a highly fractionated derivative of the original basalts. A closer look at the Skye volcanic rocks reveals two contaminated suites located in the upper left and lower right portions of Fig. 1. Carter *et al.*[30] suggest that the Skye volcanic rocks tapped a depleted mantle source but we suggest that perhaps the source was heterogeneous (that is, enriched and depleted).

Uncontaminated continental basalts, therefore, reflect dual mantle sources with both a time-integrated light REE depletion (Sm/Nd > the bulk earth value) and light REE enrichment (Sm/Nd < the bulk earth value). Similarly, careful investigation of contaminated suites of basalts in continental regions may provide evidence of enriched and depleted mantle regions. Basalt and nodule data are compatible with a grossly heterogeneous sub-continental mantle.

Table 1 ^{87}Sr/^{86}Sr and ^{143}Nd/^{144}Nd isotopic analyses of diopsides from micaceous garnet lherzolites found in kimberlite pipes

Locality	Rb/Sr	^{87}Sr/^{86}Sr	^{143}Nd/^{144}Nd*
Bultfontein B10 diopside	0.006	0.70646 ± 10	0.51216 ± 3
Bultfontein B10 garnet	0.315	0.70657 ± 8	—
Bultfontein B21 diopside	0.001	0.70537 ± 5	0.51237 ± 2
Bultfontein B69 diopside	0.001	0.70536 ± 9	0.51244 ± 3
Bultfontein B70 diopside	0.031	0.70702 ± 5	0.51195 ± 3
Bultfontein 017 diopside	—	—	0.51257 ± 2
Bultfontein 317 diopside	0.005	0.70722 ± 5	0.51206 ± 5
Kimberley CK26 diopside	0.015	0.70583 ± 5	0.51223 ± 2
Kimberley CK26 mica	3.723	0.70569 ± 6†	0.51185 ± 7
Kimberley CK27 diopside	0.011	0.70465 ± 8	0.51250 ± 3
Kimberley CK27 mica	7.43	0.72226 ± 10†	0.51224 ± 10
Kimberley CK32 diopside	0.003	0.70752 ± 7	0.51195 ± 4

* Normalised to ^{146}Nd/^{144}Nd = 0.7219.

† Corrected for radiogenic Sr growth for ~75 Myr, the approximate pipe emplacement age. $87_{\lambda} = 1.42\times10^{-11}$ yr^{-1}.

The compatibility of uncontaminated basalt data with mantle peridotite isotopic data indicates that perhaps these garnet and spinel lherzolites are typical of the source rocks of LIL enriched continental volcanic rocks. Trace element analyses of these peridotites, however, reveal an insufficient quota of LIL elements necessary for the production of basaltic and alkaline magmas[6,7,31] It is generally agreed that the source is an aluminous peridotite and, that the majority of garnet and spinel lherzolites have a trace element content that can, after addition of a LIL element enriched fluid, produce alkaline magmas. We have shown elsewhere[12] that ≈5% metasomatism increases the trace element content of the source such that alkaline magmas

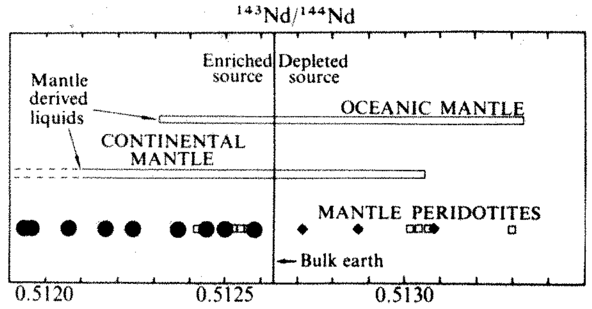

Fig. 2 $^{143}Nd/^{144}Nd$ variation in oceanic and continental basalts in relation to the mantle heterogeneity observed in diopsides from kimberlite (●) and alkali basalt nodules (refs 18, 24 and M.A.M., unpublished data) (□). Whole rock kimberlite nodule[13] data (◆) are included for comparison.

can be extracted by 5–20% melting. Other authors[32–34] have suggested that infiltration of metasomatic fluids provide the requisite amount of LIL elements and volatile fluxes that trigger anatexis. Therefore mantle peridotites containing accessory phases (amphibole, apatite, mica, sphene, and so on) are considered to be potential source materials. The diopsides analysed in this study are separated from multiply metasomatised garnet lherzolites containing both primary and secondary micas[8]. The metasomatised nature of the nodules is compatible with the isotopic data as metasomatism tends to lower the Sm/Nd ratio of the source region. Therefore phases with low Sm/Nd ratios would tend to record this enrichment event. (For comparison depletion events tend to increase the Sm/Nd ratio of the residual peridotite.)

In general there is clear evidence of recent or subrecent LIL and light REE enrichment of a metasomatic nature in the mantle source regions of oceanic and continental basalts (Fig. 1), but in the Nd and Sr isotopic data presented here we see the effects of ancient enrichment events in the sub-continental mantle. These metasomatic events are believed to be genetically related to upwelling of mantle plumes or blobs. Isotopic evidence for recent enrichment events is found in continental and ocean island basalts (Figs 1 and 2) that have $^{87}Sr/^{86}Sr$ lower than and $^{143}Nd/^{144}Nd$ ratios greater than, the bulk earth value. This indicates derivation from source regions with a time-integrated light REE depletion (Sm/Nd greater than the bulk earth value). A depleted source contrasts markedly with the LIL enriched nature of the basalts and can best be explained by a recent enrichment event in the source region. This event presumably occurred so close to the time of eruption that it has not, as yet, been recorded in the $^{143}Nd/^{144}Nd$ ratio. Such recent events may have also affected the source regions of continental basalts from Nunivak island, Alaska, the Ataq diatreme, South Yemen[12], and the Eifel rift valley, Germany (Fig. 1). We have demonstrated elsewhere[12,24] that in the case of Ataq recent enrichment or metasomatism is a precursor to continental alkaline magmatism. Rb–Sr data on basalts from the vicinity of Ataq may indicate that the event occurred 30–40 Myr before eruption of the basalts. Similarly Erlank and Shimizu[20] reported an age of 148 ± 7 Myr for a metasomatic event in kimberlite nodules, that may represent a precursor to Karroo volcanism. Recent enrichment (231 ± 25 Myr) is also evident in metasomatically veined lherzolite nodules from Dish Hill, California (M.A.M., unpublished data). One can infer from this that enrichment events, or metasomatic precursors to alkaline (or tholeiitic) magmatism represent recent mantle events ($\leq$250 Myr) superimposed on ancient, depleted, sub-continental mantle.

Enriched source regions resulting from such mantle enrichment (and associated diapirism) may temporarily be stored in the sub-continental mantle and adhere to the base of the continental (or oceanic) lithosphere[3]. Isotopic ageing will result producing the gross heterogeneities evident in the sub-continental lithosphere. However, note that these heterogeneities may result from original regional heterogeneities in the mesosphere[3] if the plumes are derived from the mesosphere, or from mixing of mesospheric (lower mantle) and upper mantle components[37]. Stored plumes may eventually be reactivated giving rise to basalts with radiogenically enriched $^{87}Sr/^{86}Sr$ ratios and $^{143}Nd/^{144}Nd$ ratios that reveal a time-integrated light REE enrichment in the source region (Sm/Nd < the bulk earth value). Such enriched sources, that are ancient in age, are found primarily below continents (Fig. 1) and are volumetrically insignificant in the oceanic mantle. For example, we find trivial amounts of undifferentiated mantle as plumes below only two oceanic islands (see refs 35, 36), whereas melting of ancient enriched mantle is more common in the source regions of continental flood basalts and other continental volcanic rocks (Fig. 1). Furthermore the relative predominance of enriched mantle in sub-continental regions complements the depleted nature of the oceanic mantle. Such a balance must exist somewhere if the assumption of a planetary distribution for the bulk earth is correct.

Conclusions

Hence, diopsides separated from kimberlite peridotite nodules exhibit a considerable range in Sr and Nd isotopes extending the mantle array to radiogenic $^{87}Sr/^{86}Sr$ values. It is evident from consideration of these data, and unpublished data on alkali basalt nodules, that the sub-continental mantle has been subjected to various enrichment events that produce a grossly heterogeneous mantle. The bulk of the sub-continental mantle may be depleted (as shown by the increase in Sm/Nd ratio) during continental formation, resulting in very old ages in most anhydrous residual nodules. However, the gross heterogeneity may result from the superposition of recent ($\leq$200 Myr) and in some cases ancient enrichment events (for example, a decrease in Sm/Nd ratio) on the already depleted mantle. Regions of the mantle sampled by alkali basalts and kimberlites will therefore be characterised by both a time-integrated light REE depletion and light REE enrichment.

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γ-β-Thalassaemia studies showing that deletion of the γ- and δ-genes influences β-globin gene expression in man

L. H. T. Van der Ploeg, A. Konings[||], M. Oort[*], D. Roos[†], L. Bernini[‡] & R. A. Flavell[§]

Section for Medical Enzymology and Molecular Biology, Laboratory of Biochemistry, University of Amsterdam, Jan Swammerdam Institute, PO Box 60.000, 1005 GA Amsterdam

* Scheperziekenhuis, Emmen, The Netherlands. † Centraal Laboratorium voor de Bloedtransfusiedienst, Plesmanlaan 125, Amsterdam, The Netherlands. ‡ Laboratorium voor Anthropogenetica, Sylvius Laboratorium, State University Leiden, Leiden, The Netherlands

In γ-β-thalassaemia, human γ- and β-globin gene expression is suppressed; this results in a severe anaemia in newborns which subsequently develops into a β-thalassaemia syndrome in adult life. This hereditary disease is now shown to be the result of a deletion of at least 40,000 base pairs of the $\gamma\delta\beta$-globin gene locus. The γ- and δ-globin genes are deleted in the affected chromosome but, surprisingly, the β-globin gene is still present, together with a large segment of the DNA sequences flanking the gene on its 5'-side and the entire region on the 3'-side of the gene. Hence, a deletion of DNA far from the β-globin gene results in the suppression of its activity.

THE human non-α-globin genes are clustered in a short region of chromosome 11. During fetal life, the major haemoglobin expressed is HbF ($\alpha_2\gamma_2$) where the γ-chains are coded for by two non-allelic γ-globin genes (Gγ and Aγ). At about the time of birth, the expression of the γ-globin genes gradually ceases and HbF is replaced by HbA ($\alpha_2\beta_2$) and a low level of HbA$_2$ ($\alpha_2\delta_2$) (ref. 1). The fetal and adult genes are closely linked and gene mapping studies using Southern blotting[2] and the analysis of cloned genes have established the physical linkage of the δ- and β-genes[3,4], the two γ-genes[5] and the tie up between these maps to describe the linkage between these four genes[6–8]. Figure 1 shows the physical map of the β-related globin genes.

Several well-defined defects in the functioning of these genes have been described. In addition to the abnormal haemoglobin proteins (such as HbS in sickle cell anaemia), inherited diseases collectively called the thalassaemias have been described in which the level of expression of these genes is altered. In the most common type, β-thalassaemia, two forms of the disease can be distinguished. In β^+-thalassaemia, reduced amounts of β-globin are produced as a result of low levels of globin messenger RNA (mRNA)[1]. In the second type, β^0-thalassaemia, no β-globin is produced. β^0-Thalassaemia is a heterogeneous disease and except for two forms of this disease[9–11] the molecular basis is unknown. The rarer disease, $\delta\beta^0$-thalassaemia, is the result of a gene deletion (see ref. 1 for references) which inactivates both the δ- and β-globin genes. The deletion maps from the intervening sequence of the δ-globin gene[6,12,13] to a site 1,800 base pairs past the β-globin gene[13]; altogether, 10,000 base pairs have been deleted[13]. Finally, in the condition HPFH (hereditary persistence of fetal haemoglobin) the entire $\delta\beta$-globin gene region has been deleted (see ref. 1 for older references), spanning from sites 4 kilobase pairs in front of the δ-globin gene in two cases[6,7] and 7 kilobase pairs in front of the δ-globin gene in another (R. Bernards and R.A.F., unpublished) to a position well past the β-globin gene.

Several other rare forms of thalassaemia affecting the β-related globin genes are known. In γ-β-thalassaemia, first described by Kan *et al.*[14], a severe anaemia is evident in newborns as a result of a reduction of the γ/α synthetic ratio to about 0.5. As γ-chain synthesis is switched off in the course of normal development, the disease develops into a mild β-thalassaemia which is unusual in that the HbA$_2$ ($\alpha_2\delta_2$) levels are normal, rather than elevated as in classical β-thalassaemia. To explain this, Kan *et al.*[14] postulated that the entire $\gamma\delta\beta$-region has been deleted in γ-β-thalassaemia.

We show here that the Gγ-, Aγ- and δ-globin genes have been deleted in γ-β-thalassaemia. Surprisingly, however, the β-globin gene is present, together with 2,500 base pairs on its immediate 5'-side and the entire 3'-extragenic region. Figure 1 compares the structure of the affected region in γ-β-thalassaemia with the corresponding region of normal DNA.

γ-β-Thalassaemia in a Dutch family

One of us (M.O.) has been involved in the treatment of a Dutch family which exhibits the same clinical symptoms as those originally described for γ-β-thalassaemia[14]. These data (including an extensive family study) will be presented in detail else-

Table 1 Haematological data and β/α specific activity ratios for γ-β-thalassaemia

	Patient 2	Normal females
Hb (g dl^{-1})	9.7	12.0–15.0
Erythrocytes ($\times 10^{12}$ l$^{\times 1}$)	4.5	3.5–5.5
Mean cell volume of erythrocytes (fl)	67	82–94
Reticulocyte count (%)	5.2	0.5–1
HbA$_2$ (% of total Hb)	3.1	1.9–3.0
HbF (% of total Hb)	1.0	0.1–1.5
β/α synthetic chain ratio	0.49	0.9–1.0

Haemoglobin was determined by the cyanomethaemoglobin method, the erythrocytes counted in a Hycell counter and the mean cell volume of erythrocytes calculated from the erythrocyte count and the haematocrit. The reticulocyte count was determined by microscopy and the HbA$_2$ and HbF determined as described in refs 20 and 21. The β/α synthetic ratios were determined by the incorporation of ^{3}H-leucine by reticulocytes followed by the separation of the globin chains according to ref. 22. The specific activity of each chain was determined by hydrolysing the α- and β-globins and determining the leucine content directly. The values for normal females are the average values found in the laboratories of D.R. and L.B.

[§] To whom correspondence should be addressed at the National Institute for Medical Research, Laboratory of Gene Structure and Expression, The Ridge Way, Mill Hill, London NW7, UK.

[||] Present address: Erasmus University, Medical Faculty, Department of Cell Biology and Genetics, Rotterdam, The Netherlands.

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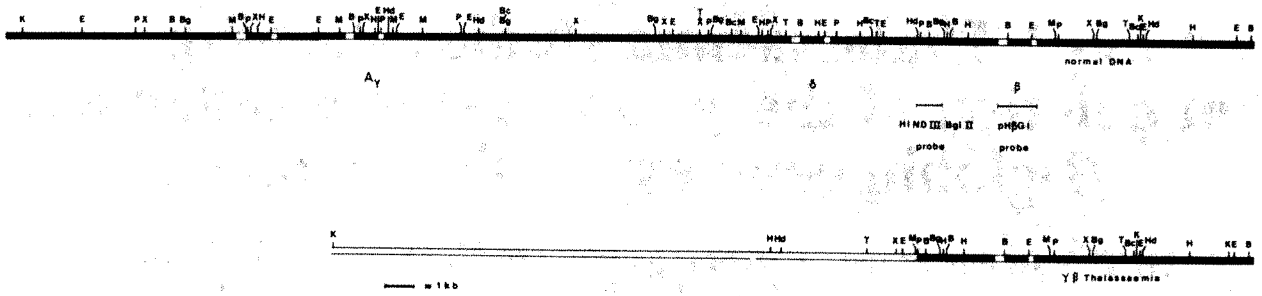


Fig. 1 Restriction map of the γ-β-thalassaemic globin genes compared with normal DNA. The probable positions of the coding regions are shown as open boxes. It should be stressed that only extragenic sites which can be detected for a given enzyme by the blotting analysis are those closest to the sequence complementary to the probe used. Certain sites between the δ- and β-globin genes are as originally described in ref. 6. The positions of the remaining sites are described in refs 8 and 13, and most were originally described in refs 3 and 5. The *Msp*I sites are from L.H.T.V.P. and R.A.F. (unpublished). The *Kpn*I site to the 5'-side of the Gγ-globin gene has been remapped at 7.5 kilobases upstream from the gene in *Hpa*I + *Kpn*I double digests. This means that the *Kpn*I fragment is 39 and not 46 kilobases, as estimated from its electrophoretic mobility in agarose gels[8]. B, *Bam*HI; Bc, *Bcl*I; Bg, *Bgl*II; E, *Eco*RI; H, *Hpa*I; Hd, *Hin*dIII; K, *Kpn*I; M, *Msp*I; P, *Pst*I; T, *Taq*YI; X, *Xba*I.

where. Briefly, of a family with 13 progeny, 7 (now adults) were found to exhibit a β-thalassaemia minor with normal HbA_2 levels rather than the elevated HbA_2 characteristic of classical β-thalassaemia. Two of these adults have been analysed in the present article (patients 2 and 3). Some of the relevant haematological data for patient 2 are compared with the average data for normal individuals in Table 1. In particular, note that the β/α chain synthetic ratio was 0.49 in this case, rather than the 0.9–1.1 values found for normal individuals.

In the past 14 yr, nine infants, progeny of the seven affected individuals described above, have been examined by M.O. They showed the clinical course described by Kan *et al.*[14] with severe postnatal haemolytic anaemia, a mixed hyperbilirubinaemia with both direct and indirect bilirubin being elevated; a drastic decrease in haemoglobin level occurred in the immediate postnatal period.

During postnatal development, a chronic microcytic anaemia persisted in all cases, with normal or near normal reticulocyte counts and bilirubin levels. The globin synthetic ratios have been measured in two affected infants, namely, a 4-month-old child of patient 2 and a newborn child of patient 3. In the first case the child showed 23% HbF, with a β/α ratio of 0.56 and a γ/α ratio of 0.74. In the second case 87% HbF was found, with a γ/α synthetic ratio of 0.5. Thus, γ- and β-gene expression is impaired in the same way as originally described by Kan *et al.*[14]. As the γ-lesion has only been observed in several children of the sibling adults with the β-thalassaemia trait (whose spouses were in all cases haematologically normal), there can be no doubt that the γ-β-thalassaemia lesion is carried on the same chromosome. (Normal infants have also been born in these families with neither γ- nor β-globin gene expression affected.)

Abnormal β-globin gene fragments in γ-β-thalassaemia

To analyse the globin gene structure in this disease, we isolated DNA from peripheral blood from two affected adult γ-β-thalassaemia heterozygotes (patients 2 and 3) and their normal spouses (1 and 4), digested the DNA with various restriction endonucleases and analysed the globin gene fragments by Southern blotting[2], followed by filter hybridisation[2,15,16] as described previously[3,4] using cloned human β and γ-complementary DNA (cDNA)[17] as probes for the β-δ- and γ-globin genes, respectively (see Fig. 2 legend for details).

Hybridisation of *Bgl*II, *Xba*I and *Bam*HI-digested DNA from the γ-β-thalassaemia patients with γ-gene probes (pHγG1)[17] showed only the normal γ-globin bands described previously[5] (Fig. 2). This must mean either that the γ-globin gene region is normal in γ-β-thalassaemia or that the entire γ-region has been deleted in the affected chromosome. The reduced intensity of the bands in the patients compared with the controls suggested the latter. Using probes for the β-globin structural gene sequences (pHβG1 DNA)[17], abnormal bands were seen in *Hin*dIII, *Eco*RI, *Xba*I, *Msp*I and *Taq*Y-digested DNA in addition to the normal bands derived from the unaffected chromosome (Fig. 2; Table 2). In contrast, the *Pst*I, *Bgl*II (Fig. 2) and *Hpa*I (Fig. 3) patterns for the δ- and β-globin genes of the thalassaemics were the same as for control individuals (Fig. 2; Table 2 gives the relevant sizes of the fragments). The *Bam*HI patterns for the control individuals and one of the patients are also the same; they do, however, differ from all other human DNAs that we have examined in that they contain a 22-kilobase 3' *Bam*HI β-globin gene fragment instead of the usual 8.6-kilobase fragment. The second patient is heterozygous for this *Bam*HI polymorphism; this DNA contains both the 22-kilobase and 8.6-kilobase 3' β *Bam*HI fragments. This *Bam*HI polymorphism is, however, not directly relevant to the γ-β-thalassaemia lesion because it is also found in individuals unaffected by the disease.

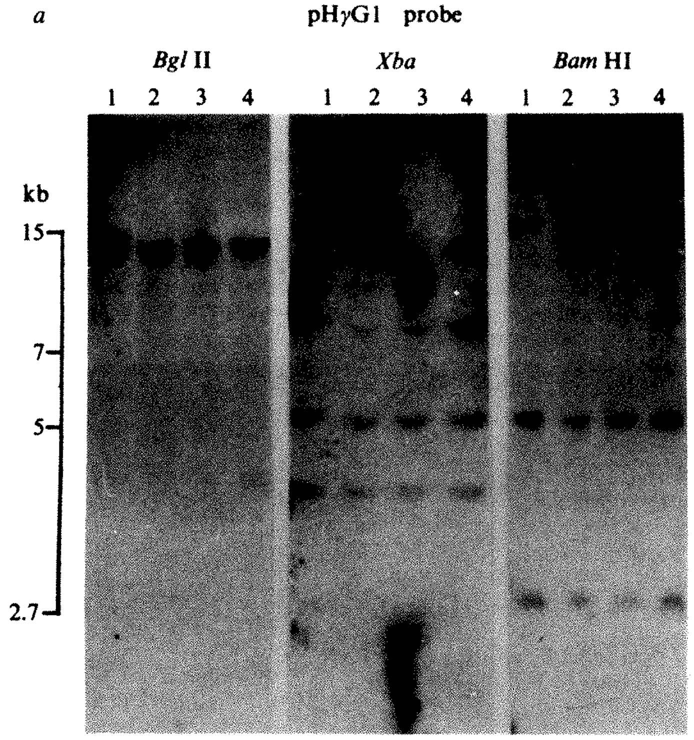
Inspection of the restriction map of the β-related globin gene locus (Fig. 1) suggests that a break has occurred in γ-β-thalassaemia in the 200-base pair region between *Hin*dIII and *Pst*I on the 5'-side of the β-globin gene: all cleavage sites on the 3'-side of this site are normal, whereas the *Hin*dIII, *Eco*RI, *Bcl*I, *Taq*YI, *Xba*I and *Msp*I sites on the 5'-side differ from the normal ones.

To confirm this, we hybridised the same DNA samples with the 0.9-kilobase *Hin*dIII + *Bgl*II fragment containing the DNA region 1.9–2.8 kilobases to the 5'-side of the β-globin gene, that is, the region of the putative break point. Figure 1 shows where this probe is located on the map. The *Bgl*II–*Hin*dIII probe detects all the abnormal 'β'-globin bands detected above and the corresponding normal β-globin fragments (Fig. 4). We have also mapped the 'abnormal' cleavage sites of those enzymes (*Bam*HI, *Hpa*I and *Pst*I) that gave apparently normal patterns with pHβGI probes, because the cleavage sites mapped with pHβGI are located between the β-globin gene and the break point in γ-β-thalassaemia. Thus, *Bam*HI gives a 0.7-kilobase band with both normal and γ-β-thalassaemia patient DNAs (Fig. 4); this 0.7-kilobase fragment is adjacent to the 1.8-kilobase *Bam*HI β-fragment at its 5'-terminus[6]. The break point must, therefore, be to the 5'-side of this (or alternatively, it is within this fragment but the new *Bam*HI site is coincidentally at the same relative position as the 'old' site). In DNA cut with *Hpa*I, a novel fragment of 5.6 kilobases is seen (in contrast to Fig. 3). DNA cut with *Pst*I still does not show any abnormal bands; only the 4.4-kilobase β-globin gene fragment is visible. We presume that we do not detect an abnormal *Pst*I fragment because the break point is essentially at the normal *Pst*I site; in this case, little or no homology would be seen between the fragment spanning the break point and our *Bgl*II-*Hin*dIII probe.

Mapping the size of the deletion in γ-β-thalassaemia

To confirm that the break point we have deduced above is indeed the correct explanation for the γ-β-thalassaemia fragment pattern, we have mapped several cleavage sites around the β-globin gene in double digests. The abnormal 5′ *Eco*RI and *Msp*I sites have been mapped in *Pst*I + *Eco*RI and *Msp*I + *Eco*RI double digests; Table 2 shows that the data from these digests fit with the position of the break point already deduced.

All the data support a model in which a break in the chromosome has occurred between *Pst*I and *Hin*dIII 2.5 kilobases upstream from the β-globin gene. To determine whether a deletion or inversion has occurred, we analysed *Kpn*I digests. In normal DNA, *Kpn*I yields a single 39-kilobase globin gene fragment containing the Gγ-, Aγ-, δ- and β-globin genes (Fig. 1, ref. 8). In DNA from the γ-β-thalassaemia patients, a second 27-kilobase band is visible which hybridises with β-globin probes but not γ-globin probes (Fig. 5). Apparently, a large deletion has occurred in γ-β-thalassaemia, eliminating the γ-globin genes entirely. The 3′ *Kpn*I site was mapped in γ-β-thalassaemia DNA in *Kpn*I + *Hpa*I and *Kpn*I + *Bam*HI double digests. To our surprise, two 3′ *Kpn*I sites are indicated in this experiment in both the patients and their normal spouses, who are also from this region of The Netherlands. The closest *Kpn*I site to the β-globin gene is 5.5 kilobases to the 3′-side of the 5′ β-globin *Hpa*I site and corresponds to the 3′ β-globin *Kpn*I site already mapped by us in several DNAs from normal Dutch, Indian and English individuals, an Italian $\text{Hb}_{\text{Lepore}}$ patient and an Italian $\delta\beta^0$-thalassaemia patient. The second site lies to the 3′-side of the *Hpa*I site downstream from the β-globin gene and,



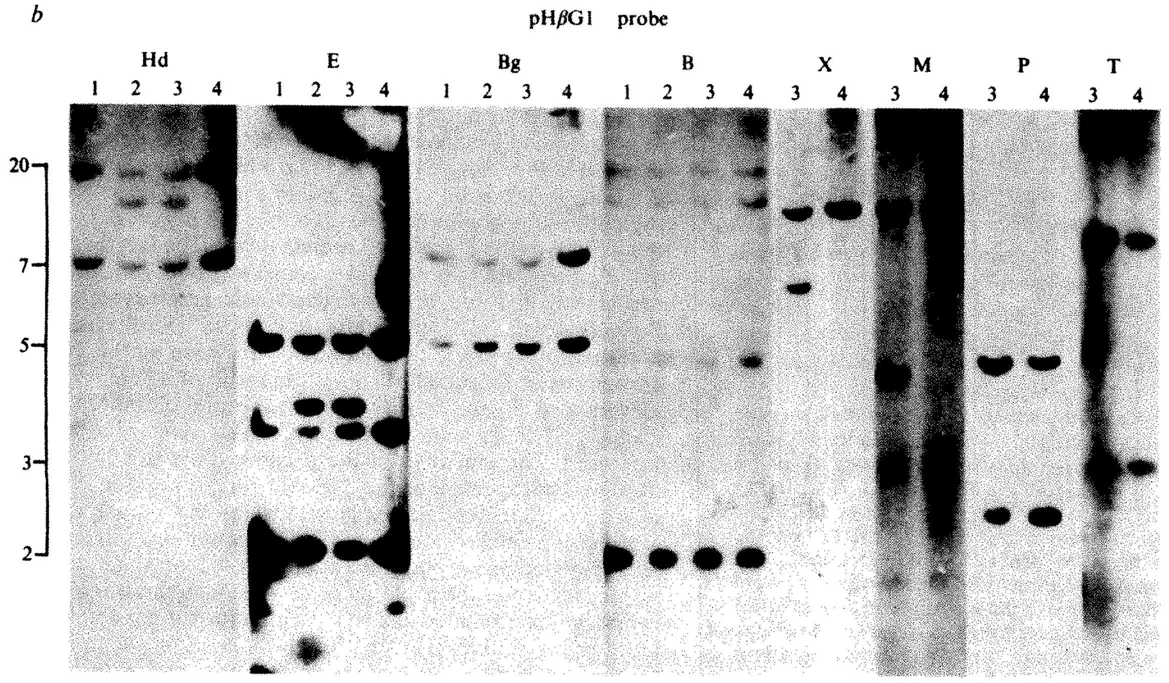


Fig. 2 γ-, δ- and β-globin gene fragments in γ-β-thalassaemia. DNA was isolated from peripheral blood of two γ-β-thalassaemic patients and their normal spouses as described[3] except that a 30-min incubation in proteinase K (100 $\mu\text{g ml}^{-1}$) in 1% SDS, 0.1 M EDTA and 0.15 M NaCl (*p*H 8.0) at 37 °C preceded the phenol extractions. DNA was digested with the relevant restriction endonucleases[3] and about 10 μg per lane was electrophoresed in 0.7% agarose gels for 36–48 h at 0.7 V cm^{-1}. The DNA was blotted[2] on to nitrocellulose filters and hybridised as previously described[3,16] except that 9% dextran sulphate (Sigma, 500,000 molecular weight) was added to the pre-hybridisation wash and to the hybridisation solution[23]. Hybridisation probes were pHβG1 (*a*) and pHγG1 (*b*) for the $\delta\beta$- and γ-genes, respectively[3,5,17]. After a 12-h hybridisation (using 1 ng of ^{32}P-labelled DNA probe per ml; 4×10^8 c.p.m. per μg) at 65 °C, the filters were washed as described elsewhere[3]. Bands were detected by autoradiography for 12–48 h as previously described[3]. For abbreviations, see Fig. 1 legend. The two additional faint bands seen in the *Bam*HI–pHγG1 hybridisation are partial digestion products.

therefore, only half the 7.7-kilobase *Hpa*I band is cut (Fig. 3). The second polymorphic *Kpn*I site is precisely located in the *Bam*HI + *Kpn*I double digest; a new 7.7-kilobase *Bam*HI + *Kpn*I double digest fragment places this site at 7.7 kilobases from the intragenic β-globin *Bam*HI site, 3.1 kilobases past the 'normal' *Kpn*I site (Fig. 3). Patient 3 and control 4 are, therefore, heterozygous for a 3′ *Kpn*I polymorphism as well as being homozygous for the 3′ *Bam*HI polymorphism described above.

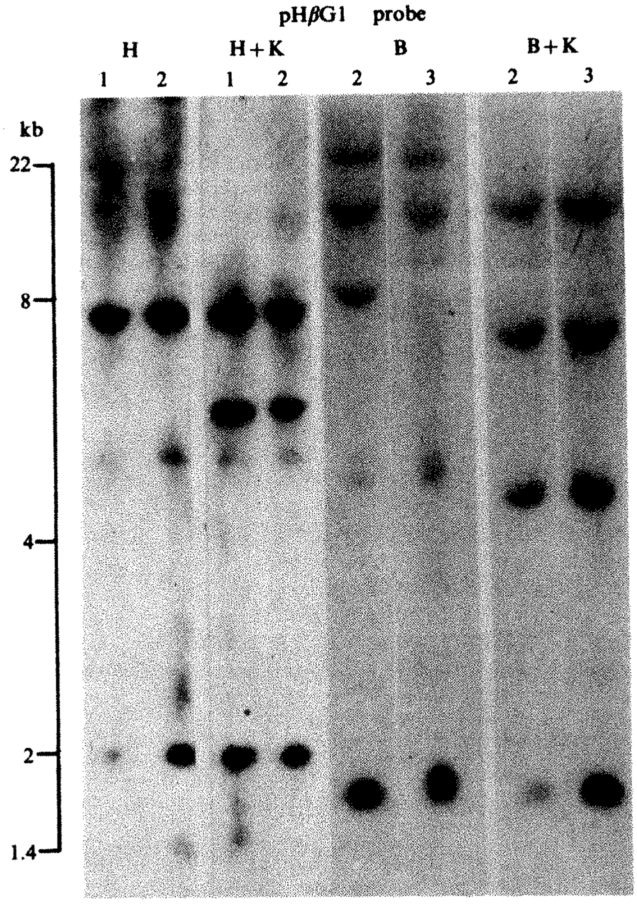


Fig. 3 Mapping the *Kpn*I sites at the 3′-side of the β-globin gene in DNA from normal individuals and γ-β-thalassaemics: a 3′ *Kpn*I polymorphism. DNA from γ-β-thalassaemics (patient 3) or his normal spouse (no. 4) was digested with *Kpn*I (K), *Hpa*I (H) or *Bam*HI (B) either single or in double digests. Only one-half of the 7.7-kilobase *Hpa*I β-fragment is cleaved by *Kpn*I to give the *Hpa*I + *Kpn*I double digest fragment of 5.5 kilobases; one half of the 22-kilobase 3′ *Bam*HI β-fragment is cleaved at the same *Kpn*I site described previously[8] to give the 4.6-kilobase double digest fragment and the other half lacks this site and is cleaved at a second *Kpn*I site 3.1 kilobases further in the 3′ direction. For abbreviations, see Fig. 1 legend.

Table 2 Size of globin gene fragments in restriction enzyme digests

	Fragment size		
Enzyme	γ-β-Thalassaemia gene	δ-Gene	β-Gene
Detected with pHβG1 probe			
*Hind*III	12.0	17.0	7.6
*Eco*RI	4.2	2.3 + 1.9	5.8 + 3.7
*Bgl*II	NAB	8.4	5.0
*Bam*HI	NAB	15.0 + 4.3	1.8 + 22 or 8.6
*Xba*I	6.4	10.8	
*Msp*I	4.5	10.5	
*Pst*I	NAB	2.3	4.4
*Taq*YI	9.0	8.6	3.1
*Pst*I + *Eco*RI	NAB	1.7 + 0.6	3.6 + 0.8
*Hpa*I	NAB	1.9 + 1.4	7.8
*Eco*RI + *Msp*I	3.7	2.3 + 1.9	5.8 + 0.8
*Kpn*I	27	39	
*Kpn*I + *Hpa*I	NAB	1.9 + 1.4	7.8 or 5.5
*Bam*HI + *Knp*I	NAB	15.0 + 4.3	7.7 or 4.6
Detected with *Hind*III–*Bgl*II probe			
*Hind*III	12.0	—	7.6
*Eco*RI	4.2	—	5.8
*Bam*HI	NAB	4.3	0.7
*Xba*I	6.4	10.8	
*Hpa*I	5.8	—	3.1
*Kpn*I + *Hpa*I	5.8	—	3.1

The γ-β-thalassaemia fragments are from the present paper. The β- and δ-globin gene fragments have been reported previously[3,6,8,13]. NAB, no additional bands.

Figure 1 compares the maps of the normal globin gene region with that of γ-β-thalassaemics. How large is the deletion in γ-β-thalassaemia? The minimal size of the deletion seems superficially to be 12 kilobases, that is, the difference in size between the normal *Kpn*I fragment (39 kilobases) and the abnormal fragment (27 kilobases). Inspection of the map in Fig. 1 shows, however, that this cannot be the case. This would place the 5′-break point in the γ-globin gene region and would result in abnormal *Bam*HI, *Xba*I and *Bgl*II γ-globin gene fragments; these are not seen. The explanation must, therefore, be that the deletion extends past the *Kpn*I site on the 5′-side of the γ-globin genes; the entire γ-globin gene region is deleted in γ-β-thalassaemia. *Hpa*I digests of DNA from γ-β-thalassaemics define the minimal size of the deletion. Because the γ-globin genes are completely deleted in γ-β-thalassaemia, the first *Hpa*I site present on the 5′-side of the break point of the deletion must be past both γ-globin genes. Fritsch *et al.*[6] have shown that the first *Hpa*I site to the 5′-side of the Gγ-globin gene is 23 kilobases upstream from the gene in normal DNA and we have confirmed this in several normal individuals. Let us assume that this is the 5′ *Hpa*I site of the abnormal 5.6-kilobase *Hpa*I fragment in γ-β-thalassaemia. At its 3′-end the 5.6-kilobase fragment must contain the 0.9 kilobases of DNA which extend from the 3′-break point of the deletion to the *Hpa*I site in front of the β-globin gene. The closest position for the 5′-break point of the deletion is, therefore, 4.7 kilobases (5.6–0.9) from the '5′ Gγ' *Hpa*I site; inspection of Fig. 1 shows that this predicts a minimal deletion of 42 kilobases. Maniatis and his colleagues have recently shown that an embryonic globin gene is located to the 5′-side of the Gγ-globin gene; as this ε-gene is 8 kilobases upstream from the Gγ-globin gene (T. Maniatis, personal communication) we conclude that this gene is deleted in γ-β-thalassaemia.

Discussion

Comparison of the detailed maps of restriction endonuclease cleavage sites in normal DNA[3–8] and DNA from γ-β-thalassaemic patients has shown that this disease is the result of a large deletion in the γδβ-globin gene locus which has its 3′-end point 2.5 kilobases in front of the β-globin gene. The Gγ-, Aγ- and δ-globin genes are entirely deleted, but the β-globin gene and the regions to the 3′-side of β remain intact. The fact that the γ- and δ-globin genes are deleted explains why both gene products are grossly reduced in γ-β-thalassaemic heterozygotes. The δ-globin levels are apparently normal in γ-β-thalassaemia heterozygotes despite the deletion of this gene on the affected chromosome. Presumably, this is the result of a compensatory elevation of δ-gene activity from the second chromosome; in classical β-thalassaemia, δ-globin levels are also usually increased. The inactivity of the β-globin gene in adult life is, however, unexpected, as the β-globin gene is present.

We see four possible explanations for this paradox. First, γ-β-thalassaemia results from a double mutation, a deletion of the γδ-region and a point mutation or a second small deletion which inactivates the β-globin gene. Although this is unlikely it cannot yet be excluded and so remains a formal possibility. It is more probable, however, that the β-thalassaemia seen in γ-β-thalassaemics is the result of the deletion mapped in this article.

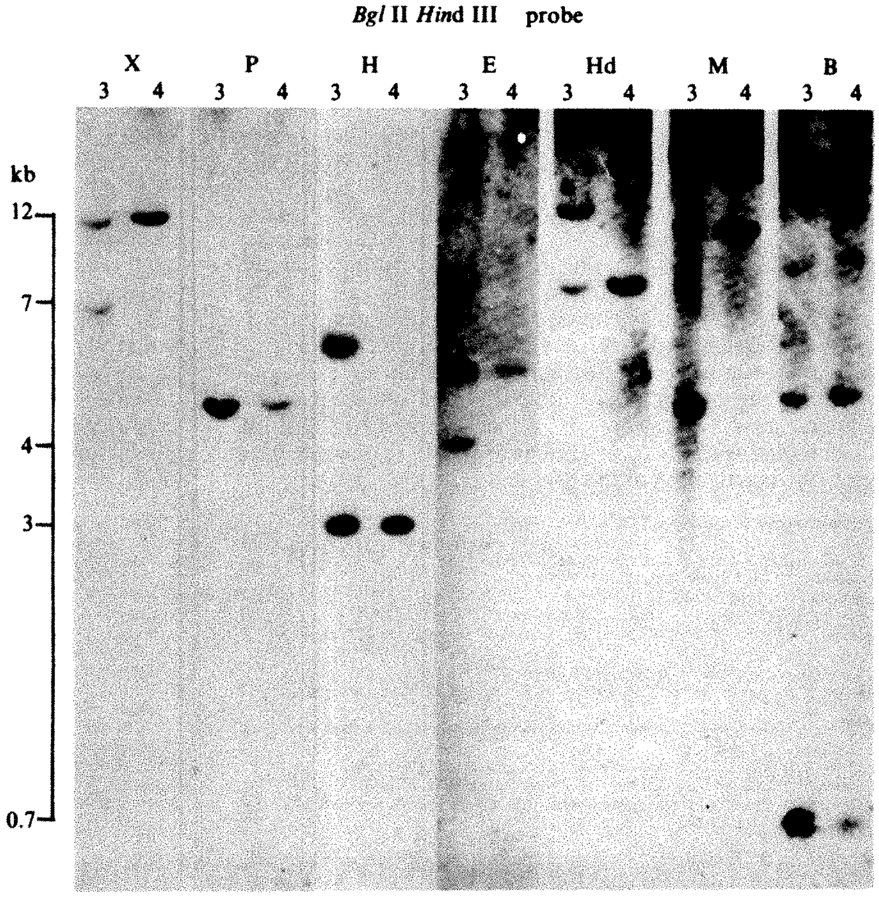


Fig. 4 β-globin gene fragments in γ-β-thalassaemia, detected with the *Bgl*II-*Hind*III probe for the 5′-extragenic regions of the β-globin gene. Filter-bound DNA from a γ-β-thalassaemic patient and his normal spouse was prepared as described in Fig. 2 legend. The *Bgl*II-*Hind*III probe was prepared from a cloned *Hind*III fragment from the β-globin gene of a β^0-thalassaemia patient (F. G. Grosveld, H. H. Dahl, E. De Boer and R.A.F., unpublished) by double digestion with these enzymes, followed by electrophoresis on a 1.2% agarose gel. The fragments were extracted according to the method of Tabak and Flavell[24] except that the DNA fragment was eluted from the hydroxyapatite with 400 µl of 0.5 M EDTA (*p*H 7.5) instead of phosphate buffer. For abbreviations, see Fig. 1 legend.

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The effect might be either *cis*- or *trans*-acting.

Second, it is possible that the reduction in β-globin gene expression is a result of the deletion mapped, but that its effect is not confined to the 'deleted' chromosome; it could be a general effect on both β-globin genes. To explain this, we could postulate that a *trans*-acting component is coded for by a gene in the $\gamma\delta$-globin gene region. The fact that the β/α synthetic ratio is 0.49 instead of 1 is, however, more in line with the inactivation of one chromosome, presumably from the one carrying the deletion, by a *cis*-acting effect; a precise reduction of β-globin gene expression by a factor of two as a result of a drop in the level of a *trans*-acting component by the same factor seems unlikely, but this is not excluded by our data.

How do we explain the lack of β-globin gene activity by a *cis*-acting effect of the deletion? We could argue that the transcription unit of the β-globin gene extends into the DNA that has been deleted; conceivably the promoter or another regulatory sequence would be located more than 2.5 kilobases to the 5′-side of the β-globin gene. This is unlikely; a rabbit β-globin gene containing only 1.4 kilobases of the sequences upstream from the 5′-end of the gene is transcribed to give active globin mRNA when introduced into mouse L cells by transformation[18], there is no reason to believe that the homologous human gene would do otherwise. We have also mapped the unspliced rabbit β-globin pre-mRNA to be a transcript of the mRNA coding regions plus the two intervening sequences; the 5′-extragenic sequences are not present on this transcript[19].

The final explanation is that sequences far from the β-globin gene also influence its activity in *cis*. For example, a DNA sequence in the $\gamma\delta$-globin gene region could be essential for β-globin gene activity. Specifically, we have considered the possibility that the $\gamma\delta\beta$-region is split into chromosomal domains containing the fetal (Gγ and Aγ) and adult (δ and β) globin genes, respectively[12]. DNA sequences upstream from the δ-globin gene might be necessary to organise an active configuration for the whole $\delta\beta$-region; their deletion would render this impossible and hence inactivate the β-globin gene,

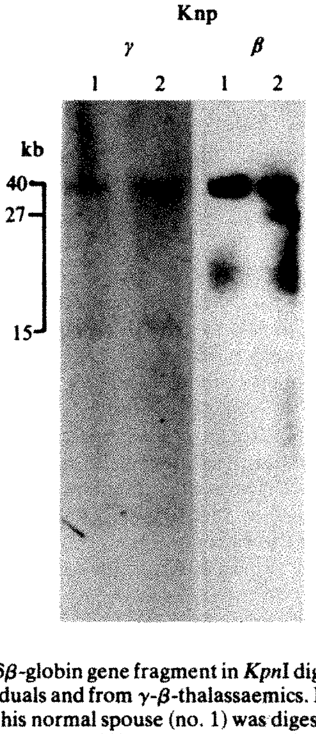

Fig. 5 the $\gamma\delta\beta$-globin gene fragment in *Kpn*I digests of DNA from normal individuals and from γ-β-thalassaemics. DNA, 10 µg, from patient 2 and his normal spouse (no. 1) was digested with *Kpn*I and electrophoresed on a 0.5% agarose gel for 72 h at 1 V cm^{-1}. The DNA was blotted, hybridised to either pHγGl or pHβGl DNA and further processed as described in Fig. 3 legend. The γ-probe filter was washed only to 0.3 × SSC to detect possible abnormal embryonic globin gene fragments. The faint 15-kilobase band contains the embryonic ε-genes and the 39-kilobase band contains the Gγ-, Aγ-, δ- and β-globin genes (see Fig. 1, ref. 8). Note that the 27-kilobase fragment in the DNA from the γ-β-thalassaemic patient only hybridises with the β-probe.

even though it would remain present on the chromosome. If these putative sequences were closely linked to the δ-globin gene and if they were essential for β-globin gene expression, the presence of the δ-globin gene in the population would be selected for by this linkage. This might explain why the apparently functionless δ-globin gene (which only contributes about 3% of the adult non-α-globin chains) is maintained in the human gene pool. It is also conceivable that some types of classical β-thalassaemia might be the result of mutations in the same DNA sequences discussed here, that is, further from the β-globin gene than has been hitherto considered.

Alternatively, the inactivation of the β-globin gene in γ-β-thalassaemia might be the result of a 'position effect' of the type previously described in *Drosophila*; here, the juxtaposition of a gene and a segment of heterochromatin results in the inactivation of the gene. Conceivably, the 5′-break point of the deletion in γ-β-thalassaemia is in a segment of heterochromatin which causes the inactivation of the β-globin gene when translocated to a position next to the gene.

The existence of sequences influencing gene expression in eukaryotes over large distances has its precedents. The mapping of distal DNA sequences in the human β- and δ-globin gene regions, which influence the expression of the γ-globin genes, has recently been described by ourselves[13] and by others[6,7]. In the latter cases, however, the putative regulatory sequences repress γ-globin gene expression in adult life; their deletion results in the persistence of γ-globin gene expression. In the case described here, the opposite is seen, namely, regulatory sequences are required to activate β-globin gene expression. Hopefully, the analysis of other rare types of thalassaemia may further identify DNA regions involved in the expression of the human fetal and adult globin genes.

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LETTERS

Test of the inverse Compton model for Cygnus X-1

E. P. T. Liang

Institute of Theoretical Physics, Stanford University, Stanford,
California 94305

The X-ray spectrum of Cygnus X-1 from ~1 keV to ~200 keV exhibits two distinct components. The spectrum below ~10 keV is soft and may be the tail of comptonised black body if the turnover at ~1 keV is real, while that above ~10 keV is a simple power law[1,2]. The spectra above 200 keV remain uncertain with some showing a cutoff but others a continuation of the power law[3]. The power law hard spectrum is thought to be the result of unsaturated Compton up-scattering of soft photons by hot thermal electrons[4]. The comptonisation region may be the optically thin inner part of the disk or spherical accretion flow near the black hole or a hot corona surrounding a thin disk[1]. The unsaturated Compton model is attractive as it accounts for the power law spectrum with index $\alpha \sim 1$ ($I_\nu \propto \nu^{-\alpha}$) and the alleged high electron temperature ($T_e > 10^9$ K). However, there are still questions about the origin of the soft photon supply and stability. As important conclusions are drawn from this model (for example, interpretation of the high energy cutoff as a measure of the electron temperature and α as a measure of the Komponeet parameter $y \equiv (4kT_e/m_ec^2)\tau_{es}^2 \simeq 4/(3\alpha+\alpha^2)$, $\tau_{es} \equiv$ electron scattering depth)[4,5], it is crucial to have independent verification of this idea. We propose here a more critical test and show that the hard X-ray data available to date strongly support this model.

A steady supply of soft photons of mean energy $h\nu_0$ and luminosity L_i injected into a comptonising medium of Komponeet parameter y will emerge with luminosity $L \sim L_i\,e^y$ at least for $y \leqslant 1.5$ (unsaturated comptonisation, for saturated comptonisation $y \gg 1$ and $L \sim L_i 3kT_e/h\nu_0$; see refs 5, 6 for numerical results of intermediate regimes). L has two spectral components: a power law of total luminosity L_h for photons with many scatterings and a residual soft component of luminosity L_s for photons with few scatterings. Using photon number conservation one finds

$$L_s = L_i\left(1 - q\frac{h\nu_0}{kT_e}e^y\right)\left(1 - \frac{qh\nu_0}{kT_e}\right)^{-1};$$

$$L_h = L_i(e^y - 1)\left(1 - \frac{qh\nu_0}{kT_e}\right)^{-1}$$

where $q \sim 0(1)$ depends weakly on y and spectral details of L_i. Hence for $h\nu_0 \ll kT_e$, we have the important prediction

$$L_h/L_s = e^y - 1 \quad \text{for} \quad y \leqslant 1.5 \tag{1}$$

We now consider whether the large body of spectral data of Cyg X-1 reported in the past 15 yr is consistent with equation (1), assuming that we can identify L_s with the 2–10 keV luminosity and L_h with the 10–200 keV luminosity. Whether the 2–10 keV luminosity itself involves some comptonisation of even softer photons with electrons of lower T_e and y should not affect this test. It is assumed in the above identification that the (highly uncertain) luminosity below ~1 keV originates from a spatially separate region and is unrelated to that above 10 keV.

The sharpest test of equation (1) comes from the 'transition' data when Cyg X-1 exhibited anticorrelated variations in soft and hard X-ray luminosities, with the pivoting point at ~10 keV. We can find published simultaneous soft and hard X-ray data for only three such events (March 1971, May 1975, November 1975–February 1976), although there must be others (see ref. 8). As crude estimates we simply extrapolate published spectra from 2 to 10 keV for L_s and from 10 to 200 for L_h.

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Table 1 Comparison of observed luminosity ratios with inverse Compton model predictions for Cyg X-1

Transition (and refs)		Derived from published data*				L_h/L_s predicted from Compton model $=e^y-1$ for $y\leqslant1.5$ see ref. 5 for $y>1.5$	$(L_h+L_s)/y^{1/2}$
		2–10 keV luminosity $L_s/1.1$ $\times10^{36}$ erg s^{-1} (with assumed distance of 2.5 kpc)	10–200 keV luminosity $L_h/1.1$ $\times10^{36}$ erg s^{-1} (with assumed distance of 2.5 kpc)	Komponeet parameter y	L_h/L_s		
March 1971[10]	High	12	~19†	~1.85†	~1.6	~5.4	~22.8
	Low	5	35	2.58	7.0	7.6	24.9
May 1975[11,12]	High	10	10	0.54	1.0	0.7	27.2
	Low	7	21	1.32	3.0	2.7	24.4
November 1975 to February 1976[8,13,14]	High	~12‡	9.5§	0.72	~0.8	1.0	~25.3
	Low	~7‡	19.5§	1.19	~2.8	2.3	~24.3

* Errors for L_h and y listed in Table 2. Errors for L_s cannot be estimated.

† Large reported error.

‡ Only 3–6 keV band data available[13], but they agree with extension of power law spectra from above 10 keV. Large error possible.

§ High state is average of refs 8 and 14. Low state is average of pre-November 1975 and post-February 1976 data of ref. 8

Table 2 Hard X-ray data for Cyg X-1 (1966–79)

	Reported photon number spectrum $N_E=A(E/\text{keV})^{-\beta}$ cm^{-2} s^{-1} keV^{-1}		10–200 keV luminosity		Observation date (and refs)
	A	β	$L_h/1.1\times10^{36}$ erg s^{-1}	$y=\dfrac{4}{\beta^2+\beta-2}$	
(1)	3.58	1.93 ± 0.2	$14.0^{+16.8}_{-7.4}$	$1.09^{+0.38}_{-0.23}$	September 1966[15]
(2)	$3.85^{+2.98}_{-1.71}$	1.75 ± 0.2	$30.5^{+7.7}_{-5.7}$	$1.42^{+0.63}_{-0.35}$	May 1967[16]
(3)	4.57 ± 0.61	1.91 ± 0.03	$19.3^{+5.3}_{-4.4}$	$1.12^{+0.05}_{-0.03}$	June 1969[17]
(4)	4.98	2.14 ± 0.2	$8.8^{+10.0}_{-4.5}$	$0.85^{+0.25}_{-0.16}$	May 1969[18]
(5)	3.54 ± 2.44	1.89 ± 0.22	$16.2^{+49.2}_{-14.0}$	$1.16^{+0.47}_{-0.28}$	April 1969[19]
(6)	5.41 ± 1.53	1.92 ± 0.10	$22.0^{+1.3}_{-2.7}$	$1.11^{+0.17}_{-0.13}$	April 1969[19]
(7)	$1.32^{+2.0}_{-0.79}$	1.6 ± 0.4	$19.2^{+22.5}_{-9.2}$	$1.85^{+4.4}_{-0.85}$	March 1971[10]
(8)	1.29 ± 0.09	1.45 ± 0.03	$34.9^{+1.9}_{-2.0}$	$2.58^{+0.20}_{-0.18}$	March 1971[10]
(9)	$1.44^{+0.60}_{-0.42}$	1.66 ± 0.15	$16.4^{+5.1}_{-3.6}$	$1.66^{+0.57}_{-0.36}$	October 1973[20]
(10)	$1.0^{+0.26}_{-0.20}$	1.51 ± 0.1	$21.1^{+4.5}_{-3.5}$	$2.23^{+0.63}_{-0.41}$	October 1974[20]
(11)	4.4 ± 1.0	1.84 ± 0.05	$24.4^{+12.1}_{-8.9}$	$1.24^{+0.10}_{-0.08}$	July 1972[21]
(12)	8.5 ± 3.4	2.01 ± 0.09	$24.5^{+20.0}_{-14.0}$	1.0 ± 0.11	July 1972[21]
(13)	$12.6^{+19.0}_{-7.6}$	2.4 ± 0.4	$8.7^{+6.3}_{-3.0}$	$0.65^{+0.35}_{-0.19}$	November 1975[14]
(14)	$2.51^{+0.93}_{-1.46}$	1.7 ± 0.2	$24.3^{+10.4}_{-6.8}$	$1.54^{+0.76}_{-0.40}$	November 1975[14]
(15)	$3.15^{+1.85}_{-1.16}$	1.8 ± 0.2	$20.5^{+8.4}_{-5.5}$	$1.32^{+0.53}_{-0.32}$	May 1975[12]
(16)	$27.9^{+16.3}_{-10.3}$	2.6 ± 0.2	$9.7^{+2.5}_{-1.7}$	$0.54^{+0.11}_{-0.08}$	May 1975[12]
(17)	0.99 ± 0.43	1.53 ± 0.10	$19.1^{+22.4}_{-11.9}$	$2.14^{+0.56}_{-0.39}$	June 1975[22]
(18)	1.85 ± 0.45	1.73 ± 0.06	$15.9^{+9.2}_{-6.4}$	$1.47^{+0.16}_{-0.13}$	July 1975[22]
(19)	2.52 ± 0.69	1.81 ± 0.07	$15.8^{+10.7}_{-7.1}$	$1.30^{+0.15}_{-0.13}$	July 1975[22]
(20)	$7.5^{+7.62}_{-3.86}$	2.21 ± 0.27	$10.3^{+3.4}_{-2.4}$	$0.79^{+0.29}_{-0.19}$	November 1975[8]
(21)	$3.1^{+1.80}_{-1.21}$	1.87 ± 0.17	$15.3^{+3.0}_{-2.7}$	$1.19^{+0.35}_{-0.24}$	November 1975[8]
(22)	$4.7^{+1.70}_{-2.65}$	1.85 ± 0.15	$25.1^{+4.0}_{-3.9}$	$1.22^{+0.32}_{-0.23}$	November 1976[8]
(23)	$4.9^{+1.70}_{-2.54}$	1.88 ± 0.15	$23.3^{+4.2}_{-3.4}$	$1.17^{+0.30}_{-0.21}$	November 1976[8]
(24)	$6.1^{+2.67}_{-1.87}$	1.89 ± 0.13	$27.9^{+4.3}_{-3.5}$	$1.16^{+0.24}_{-0.18}$	October 1977[8]
(25)	$5.1^{+1.39}_{-1.14}$	1.83 ± 0.09	$29.5^{+3.2}_{-3.1}$	$1.26^{+0.19}_{-0.15}$	November 1977[8]
(26)	$0.92^{+0.54}_{-0.34}$	1.42 ± 0.2	$28.2^{+13.7}_{-8.6}$	$2.78^{+2.87}_{-1.00}$	September 1977[23]
(27)	$2.06^{+1.21}_{-0.76}$	1.6 ± 0.1	$29.9^{+41.9}_{-17.3}$	$1.85^{+0.44}_{-0.26}$	December 1977*

* P. Nolan, unpublished HEAO 1 data.

Table 1 lists the data for the transitions. Despite expected errors they show surprising agreement with theory except for the (soft) 'high' state before the 1971 transition, whose large reported error box is still consistent with the inverse Compton model. Such agreement also strongly suggests that most of the 2–10 keV soft photons have the same primary origin as the power law comptonised photons >10 keV and do not come from a spatially detached region.

However, six data points are far from conclusive. We have selected 27 reported spectral measurements of hard X rays in the range ~20–≤200 keV with acceptable reported or apparent error margins (≤30% in α, see Table 2). Again we extrapolate all power law spectra down to 10 keV and up to 200 keV to obtain a uniform definition of L_h. Figure 1 plots observed L_h versus y. From equation (1) we expect an overall rise of L_h with y despite intrinsic variations in L_s. This is indeed borne out in Fig. 1. Of course, these data all have large experimental errors as well as detector sensitivity and limit differences. But any systematic bias is likely to be washed out by the large number of independent measurements. The qualitative increase of L_h with y in Fig. 1 is probably genuine and consistent with the inverse Compton model.

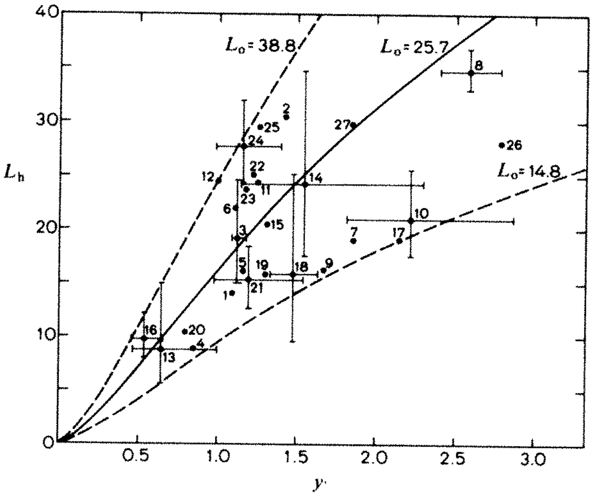

Fig. 1 Plot of L_h versus y listed in Table 2. Only a few sample error boxes are included for illustration. The solid curve represents weighted least square fit with function in equation (3). The dashed curves correspond to extremal values of L_0 sandwiching all data points. Numbers correspond to those in Table 2.

A closer look at the transition data also reveals that the variation of the combined luminosity from 2 to 200 keV is definitely correlated with y. From the limited data (Table 1 last column) we find empirically

$$(L_s + L_h) \cdot y^{-1/2} \cong \text{constant} \equiv L_0 \qquad (2)$$

This behaviour is consistent with the theoretical prediction that the accretion rate of a thin disk $\dot{M}$ onto a black hole varies as $\dot{M} \sim \text{constant} \times y^{1/2}$ because of the transonic nature of the radial drift. This result is discussed in detail elsewhere[9]. If equation (2) is valid universally then we should have (see equation (1))

$$L_h = L_0 y^{1/2}(1 - e^{-y}), \quad L_0 \approx \text{constant for } y \leq 1.5 \qquad (3)$$

A least square fit of the data with this function gives $L_0 = 25.7$ (Fig. 1). More significantly, all data points lie within a narrow wedge corresponding to $14.8 \leq L_0 \leq 38.8$ despite the wide range of L_h and y (Fig. 1).

Because of the crude nature of our data analysis and lack of calibration standard for the measurements made by different groups at different times, the above results must be viewed with extreme caution. Despite such uncertainties, the data suggest that, at least qualitatively, (1) predictions of the inverse Compton model is verified, (2) (2–200 keV luminosity $L)/y^{1/2}$ varies only slightly despite large changes in L and y. We urge that future observations should try to monitor carefully the long term variation of L_s and L_h with y simultaneously.

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Solar oscillations, stellar oscillations and cosmology

G. R. Isaak

Department of Physics, University of Birmingham,
Birmingham B15 2TT, UK

Two implications of the recent observations[1] of low angular, high radial overtones of the whole Sun are reported here. The first implication is that other main sequence stars are likely to be oscillating in similar modes and that precision spectroscopy as well as photometry from space is capable of detecting these oscillations, thereby extending seismology to stars. The second implication is a solar helium abundance $Y \leq 0.17$ with implications for cosmological models.

Recent observations[1] using the 769.9 nm resonance line of potassium of velocity oscillations of the whole disk of the Sun have resolved the broad 5-min oscillation peak into over 20 discrete spectral lines of amplitudes between 0.1 and 0.3 m s^{-1} and a nearly equal spacing of 67.80 ± 0.15 μHz. It can be readily shown that such global velocity studies could be extended to the brighter stars using large telescopes as flux collectors and such attempts have been already made[2]. Extension to fainter stars is likely to be possible using precision differential photometry from above the turbulence and variable transmission of the terrestrial atmosphere. For radial modes a rough measure of the expected photometric amplitude for a given velocity amplitude is provided[3] by $\Delta m_B \approx \Delta V/40$ where Δm_B is the blue magnitude amplitude and ΔV is the velocity amplitude in km s^{-1}. Thus for the Sun the quoted velocity amplitudes of individual frequency components imply fractional variations of 6×10^{-6} to 2×10^{-5}. Such precision has not as yet been achieved with ground-based telescopes but statistically such variations are apparently detectable at the 3σ level if more than 3×10^{11} and 3×10^{10} photons are counted or the equivalent charge is digitised. Such light fluxes are within reach of medium telescopes for stars brighter than 6 mag. Clearly, differential measurements on at

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least three stars will have to be made simultaneously to provide sufficient differential systematic stability and redundancy to identify the variable star from its comparison stars. Were the equivalent of the structure of the 5-min oscillation of the Sun to be found in stars a powerful tool of analysis of main sequence stars would become available.

The second implication follows from the interpretation of the mean spacing of 67.80 μHz with alternate $l = 0$ and $l = 1$ modes, of increasing radial order within the context of the standard model of the solar interior[4] or a model with a surface enhanced heavy element abundance[5]. Both models, if they are to agree with these observations, imply a mean heavy element abundance $Z \leq 0.004$ and a low solar neutrino flux ≤ 2.3 SNU, consistent within two standard deviations of the results of the most recent measurements[6]. The same observations and interpretation lead to a solar helium abundance of $Y \leq 0.17$, a value which agrees with that of the quiet solar wind[7]. It was already realised some 10 yr ago[8] that such a low helium abundance, among other possibilities, would lead to a solar neutrino flux consistent with the low values observed.

Thus three solar phenomena, namely oscillations, the particle wind and the neutrino flux are consistent with $Y \leq 0.17$. As hydrogen burning during the lifetime of the Sun would have increased the helium concentration and no processes are known which could have destroyed helium in the Sun, it follows that the helium abundance of the primeval material out of which the Sun formed was ≤ 0.17. This value is substantially below the mean of the values for Y obtained from other observations relating to the solar atmosphere or other objects in the Universe[9]. It is also substantially below the preferred value of current simple cosmological models[10–12] of some 0.25–0.27 even if one corrects the expectations for the new value of the neutron mean life[13]. It is possible that this model can be reconciled with a lower helium abundance by, for example, requiring sufficiently degenerate neutrinos[10–12].

A more unambiguous determination of the helium abundance of the deep solar interior may result from definitive observation and identification of the fundamental and low order radial modes of the Sun[14].

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METEOSAT observations of diurnal variation of radiation budget parameters

R. W. Saunders & G. E. Hunt

Laboratory for Planetary Atmospheres, Department of Physics and
Astronomy, University College London, Gower Street,
London WC1E 6BT, UK

Preliminary estimates of the outgoing IR flux and albedo obtained at hourly intervals, during one day, using digital METEOSAT data are reported here. The diurnal variation of these parameters varied according to the surface or cloud type over which they were being measured. The albedo over the desert, and both high and low clouds, remained constant, but altered over the sea surface. The emitted IR flux was constant over sea surfaces and low clouds, but altered over the desert during the day, due to surface heating. These diurnal variations can introduce biasing errors into time averaged radiation budget parameters, derived from polar orbiter data.

Radiation budget measurements of the Earth's atmosphere/surface system can provide information about the driving mechanisms of the Earth's atmospheric and oceanic circulation systems. Until recently, global radiation budget measurements have only been made from satellites in polar orbits[1–4,9] which sample specific areas of the Earth approximately once every 12 h. Because of the long time interval between samples and the restriction that these parameters are measured for approximately the same solar zenith angle, various assumptions have to be made when calculating daily mean values from these measurements.

The planetary albedo is determined by comparing the reflected solar radiation (measured from the satellite) with the incident solar energy calculated from the solar constant. The daily mean is inferred from one single daylight measurement by assuming the albedo is not dependent on solar zenith angle and that the radiation is scattered isotropically from all surfaces. Both of these assumptions are not valid in certain cases, such as for large solar zenith angles over the poles and for 'sunglint' reflection from the ocean surface. Sunglint occurs on the part of the Earth's surface where direct reflection from the Sun is possible as viewed by the satellite.

Table 1 The diurnal mean radiation budget parameters estimated from Polar Orbiter and Geosynchronous Orbiter data (W m^{-2})

Surface/cloud type of target area	Location of target area	Actual mean calculated from hourly METEOSAT values	METEOSAT value at 0900 h	% Difference of 0900 h value from diurnal mean
		Outgoing flux (±30* W m^{-2})		
Over desert	26° N 14° W	319	328	+3%
Over ocean	26° N 15° W	315	314	+0%
Over cumulus cloud	15° S 6° W	300	301	+0%
Over thick cloud extending up to the tropopause	26° S 35° E	214	200	−7%
Albedo (±*0.05)				
Over desert	26° N 14° W	0.32	0.32	0%
Over ocean	26° N 15° W	0.09	0.07	−22%
Over cumulus cloud	15° S 6° W	0.39	0.47	+21%
Over thick cloud extending up to the tropopause	26° S 35° E	0.59	0.62	+5%
Net flux (±*50 W m^{-2})				
Over desert	26° N 14° W	−114	−122	7%
Over ocean	26 °N 15° W	−42	−42	0%
Over cumulus cloud	15° S 6°W	−53	−58	9%
Over thick cloud extending up to the tropopause	26° S 35° E	−49	−23	53%

* The uncertainties refer to the absolute value, not to the random errors.

The outgoing long-wave radiation will have a diurnal variation over cloud-free landmasses as the ground will be heated by the incident solar radiation during the day, increasing the emitted flux. Hence twice-daily measurements from polar orbiters will not give an accurate daily mean.

Data from satellites in geosynchronous orbits such as METEOSAT are now available. These satellites are at a fixed

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point above the Equator (0° lat and 0° long for METEOSAT) and record images of the entire Earth's disk once every 30 min. This allows the radiation budget parameters to be adequately sampled for an investigation into their diurnal variation. The images are at three wavelengths, visible (0.6–0.9 μm), IR (10.8–12.2 μm) and water vapour (5.8–7.2 μm). The visible and IR channels were used to determine the albedo and long-wave flux respectively. Initially four target areas were chosen with different surface/cloud types which would be expected to have differing diurnal radiative properties. These areas were 150 km square on the Earth's surface which represents an average over at least 650 pixel values. The four areas chosen are described in Table 1, and the two cloud-free areas are shown in Fig. 1.

The visible channel has no radiometric calibration although attempts are being made to provide one[5]. For this study, a calibration was obtained by assuming the planetary (or system) albedo over North-west Africa to be 0.26 (ref. 6) at local noon on 21 August 1978. This value was based on aircraft and surface measurements taken over the area on 4 September 1974. The seasonal variation in surface albedo noted by Rockwood and Cox[6] was further south than the area chosen for the METEOSAT measurements. However, a check was made by comparing planetary albedos measured from METEOSAT at local noon in April, August and November and no significant change in albedo was noted for these months. Knowing the incident solar radiation for the 21 August, and assuming that the surface/atmosphere reflects radiation isotropically, the radiometer responses can be calibrated in terms of reflected energy. The METEOSAT visible radiometer scales linearly with incident radiation intensity, so having obtained a calibration for one radiometer value, the planetary albedo for different areas can be estimated. When the data become available, it is hoped to improve this calibration technique by a direct comparison of METEOSAT images with NIMBUS 7 ERB data and GOES EAST (70° E, 0° N) data where the images overlap.

The long-wave flux was derived from the calibration given by ESA[7] which was based on a comparison between the spacecraft digital counts and measured sea-surface temperatures. An atmospheric transmission model used for the NOAA satellite data[1] converted the 11-μm radiance to total outgoing flux at the top of the atmosphere. Figure 2 shows how the albedo and outgoing flux varied during one day (5 November 1978) for each target area. The albedo remained fairly constant over the desert, but over the sea it increased markedly with increasing solar zenith angle. This is because the sea surface can be relatively smooth and so reflects more radiation at glancing angles[8]. In contrast, cumulus cloud has a vertical structure which will reflect more of the radiation at glancing angles back in the direction from which it came. The dense high cloud gave a variable albedo which was due to changes in its structure during the day. The long-wave flux over the land increased markedly around noon due to surface heating, whereas over the sea and low cloud, the flux remained essentially constant. For high level cloud, the outgoing flux was variable which would be due to changes in cloud top altitude and structure.

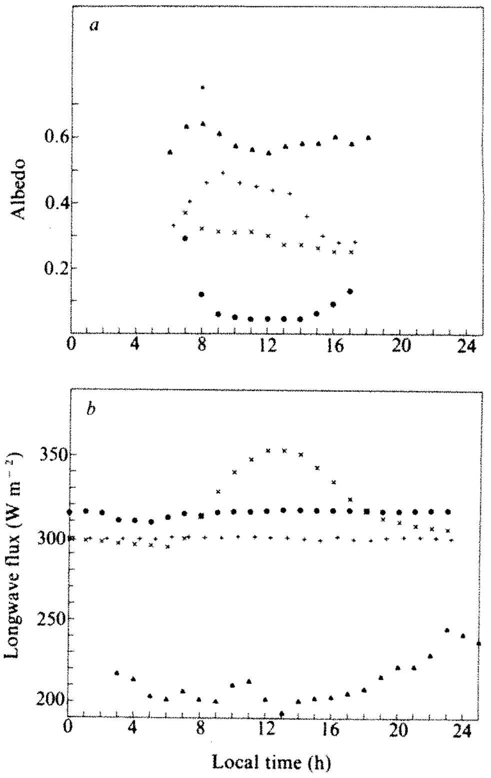

Fig. 2 The diurnal variation of albedo (*a*) and outgoing flux (*b*) as measured by METEOSAT over four surface/cloud types. ×, Over land; ●, over sea; +, over low Cu cloud; ▲, over high CuNb cloud. The absolute values of albedo and long-wave flux have an uncertainty of 0.05 and 30 W m^{-2} but the relative uncertainties will be much smaller.

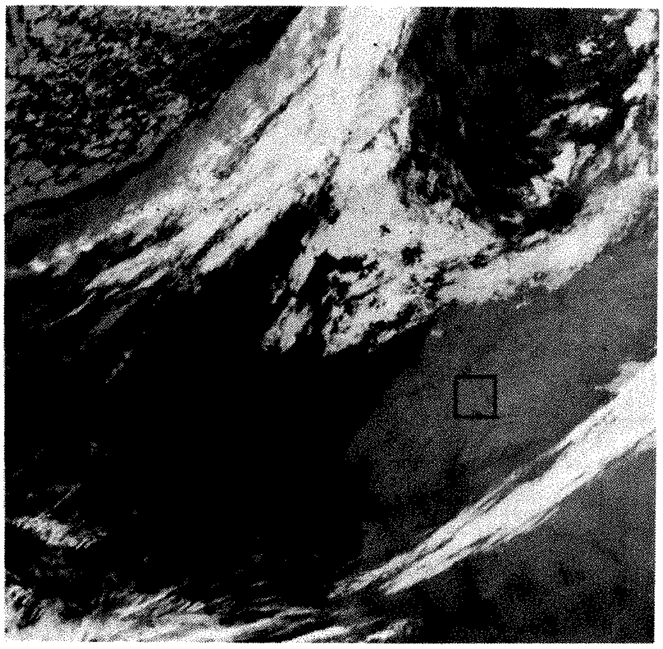

Fig. 1 The two cloud free target areas over NW Africa selected for the measurement of radiation budget parameters.

Table 1 illustrates the biasing errors, which can result from one single measurement taken from a polar orbiter; actual diurnal means are compared with the instantaneous 0900 h value using the METEOSAT observations. The latter value would correspond to the measurement made by a polar orbiter such as the NOAA series which passed over at a local time of 0900 h. The errors in the parameters, derived from the NOAA satellites, varied according to surface/cloud type. Long-wave flux errors are small and, as they only become significant over hot deserts, they are unlikely to affect the calculated global long-wave flux significantly. The albedo errors are more significant, especially as large areas of the globe are covered by ocean and low cloud; hence global albedos from polar orbiters would have a biasing error. To correct polar orbiter data sets, such as the one described by Gruber and Winston[9], the parameters over the ocean and low cloud should be corrected by the percentage differences listed in Table 1.

Also listed is the net flux which, being the small difference between two fluxes, has a large uncertainty. The errors are still present, although over the ocean, the errors are reduced because the albedo and outgoing flux errors are of opposite sign.

These results, taken from one day of METEOSAT data, show that biasing errors do exist in radiation budget parameters, obtained from polar orbiters. The geosynchronous satellite data, which are now available, allow correction factors to be determined when calculating diurnal, monthly and annual means from polar orbiter data.

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Fossil charcoal as evidence of past atmospheric composition

M. J. Cope & W. G. Chaloner

Department of Botany, Bedford College, Regent's Park,
London NW1 4NS, UK

The presence of charcoal in the geological record offers a new approach to assessing oxygen levels in palaeoatmospheres. Charcoal is formed by the incomplete combustion of woody tissues. The combustion of wood is supported by the generation of the combustible gases carbon monoxide and methane during the pyrolysis process. As the combustion ranges of these gases are restricted with decreasing oxygen availability to a level below which no combustion will occur, the production of charcoal and its appearance in the geological column constitute a record of changing oxygen availability through time. We show here that the occurrence of charcoal in rocks from the Lower Carboniferous onwards suggests that atmospheric oxygen never fell below 0.3 of present atmospheric level (PAL) during this time. Detrital charcoal formed by forest fire must have contributed an appreciable fraction to inert carbonaceous matter in sediments (kerogen) during post-Devonian time.

Natural fires have been documented in grassland, tundra, boreal forest, deciduous forest and tropical evergreen forest ecosystems in North America[1,2]. In the southeastern US, fires are common in drought-dried peat[3]. Burning may also occur in dried strand-line accumulations of both aquatic and land-derived vegetation[4].

The fossil record of terrestrial plant life indicates that small, vascular plants with upright stems had colonised the land surface by the late Silurian (~400 Myr) and that by the close of the early Devonian there were land plants at least 1 m in height and 50 mm in diameter[5]. Such plants would have been capable of forming what could loosely be called reed-swamp, or brush vegetation in drier habitats. In conditions of drought such vegetation would presumably have been capable of supporting a fire, with associated charcoal production. By late Devonian time (~350 Myr) we have records of forest trees in excess of 1 m in diameter; at this stage, forest fires, comparable with those of the present day, would have been possible. We have no evidence of terrestrial vegetation capable of fuelling any natural fire before the close of the Silurian, but presumably, before the evolution of upright terrestrial plants, accumulations of aquatic plant (algal) debris on strand lines may have presented suitable fuel for naturally occurring fire. Sizeable algae, which could accumulate on a strand line, are known from the Ordovician onwards[6] (~500 Myr). However, such strand-line accumulations of algal material would surely not ignite readily by lightning strike, and we know of no published record of charcoal of pre-Devonian age.

Charcoalified plant fragments are locally abundant in many post-Devonian sedimentary sequences. They may be identified by silky lustre and friability in hand specimen and are characterised by high reflectance in polished rock sections. Such material is known as fusinite[7]; accumulations of fusinite clasts make up the coal macrolithotype fusain[8]. Fusinite clasts are typically fragments of wood, showing well preserved cellular structure, although more rarely leaves and other plant organs can be preserved in this way[9,10]. Certain features of cell wall structure revealed by scanning electron microscope examination are particularly characteristic of a pyrolysis origin[11]. Fine anatomical details of pits and wall thickenings are preserved in charred, woody tissues, but the original components of the cell wall (middle lamella, primary wall and secondary wall) lose their intrinsic identity on pyrolysis. This apparent homogenisation of the wall takes place above 300 °C and is observed in all charcoals over a wide range of geological ages (see Fig. 1). With normal coalification of woody tissue (that is, incorporated in sediment without previous charring) the middle lamella is still visible in structured plant material up to medium volatile bituminous rank of coal[12].

Charcoal results from incomplete combustion of plant tissues[13] and is a common product of natural wildfires (forest fires)[14]. Such burning has three essential requirements: (1) a suitable fuel, (2) an ignition source, and (3) the necessary level of atmospheric oxygen to support combustion. Suitable natural ignition sources are lightning strike, volcanic activity, meteorite fall, spontaneous combustion and sparks due to rock fall. Of these, lightning strike is the most important reported cause of naturally ignited fires. Some two-thirds of all forest fires along the west coast of the US may be attributed to this cause, and 1–2% of fires in the Florida Everglades are similarly ignited[15]. The frequency of lightning discharges is estimated at 100 s^{-1} over the surface of the Earth[16]. Direct evidence of lightning strike in the geological record is rare. However, Komarek[17] notes that a petrified specimen of *Taxodium* sp. from the Miocene of Florida displayed a typical lightning streak along its length. Fulgurites result from the fusion of siliceous material (quartz sands) by lightning discharge. They are common in present day desert areas[17] and are also known from older sediments[18].

The pyrolysis of wood associated with its combustion proceeds by the evolution of water, carbon dioxide, formic acid and

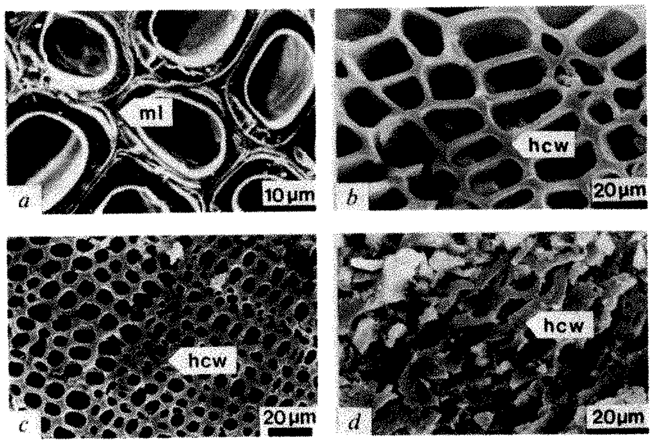

Fig. 1 Scanning electron micrographs of wood and charcoals. *a*, *Pinus sylvestris* wood showing the presence of the middle lamella between adjacent cells (ml) (scale bar, 10 µm) *b*, *c* and *d* (scale bars, 20 µm), fossil charcoals (fusinites) from the Tertiary Niederheinische Braunkohle, Jurassic Scalby Formation and Carboniferous Coal Measures respectively showing homogenisation of the cell wall (hcw) without a visible middle lamella.

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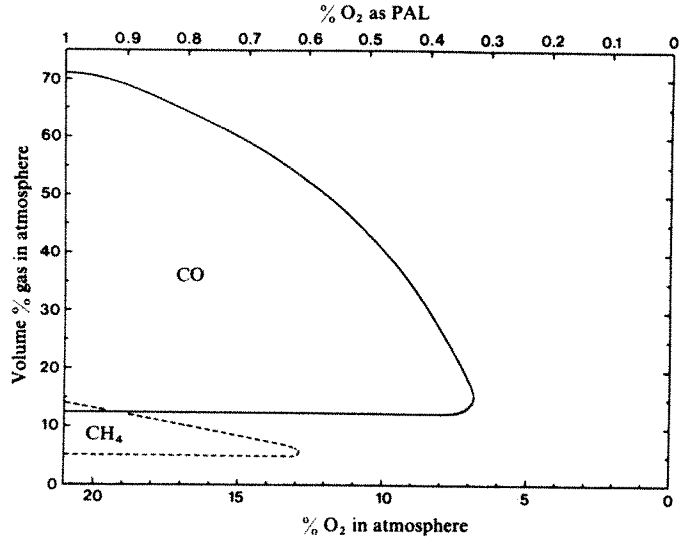

Fig. 2 Combustion ranges of carbon monoxide and methane for varying percentages of oxygen in the atmosphere (data taken from Coward and Jones[19]). As the combustion of these gases accounts for the burning associated with forest fire it is suggested here that the latter could not occur with ≤7% oxygen in the atmosphere; this corresponds to ~0.3 PAL.

acetic acid with increasing temperature up to 280 °C (ref. 13). Above this temperature, the combustible gases carbon monoxide and methane are released; it is the combustion of these gases which supports the flames constituting burning. The combustion ranges of carbon monoxide and methane are clearly defined with respect to the percentage by volume of combustible gas to air, and the limiting oxygen percentage in the atmosphere (see Fig. 2). Data from Coward and Jones[19] for combustion of various gases in air diluted by nitrogen indicate that combustion of carbon monoxide will not take place in an atmosphere with <6.8% oxygen; the corresponding figure for methane is 12.8%. It is also shown that with decreasing oxygen content of the atmosphere, the combustion range of each gas becomes more restricted. This means that at any stage of atmospheric evolution the occurrence of fire would have been limited by a threshold of oxygen availability.

Oxygen is believed to have been absent from the Earth's early atmosphere. The absence of oxygen in volcanic emissions has led to the suggestion that the accumulation of oxygen in the early atmosphere has been either by photodissociation of water in the upper atmosphere[20] or from photosynthesis by plants. Proponents of both theories recognise that oxygenic photosynthesis had become the most important oxygen producer by Phanerozoic time. Berkner and Marshall[21], favouring an initial photosynthetic build up, see a progression from 0.01 PAL of oxygen in the Cambrian to 0.1 PAL in the Silurian, and to more than PAL with the appearance of abundant land plant life in the Devonian and Carboniferous. Other workers place less emphasis on the contribution of oxygen by land plants, and stress the importance of the phytoplankton contribution. Tappan[22] sees oxygen levels from this source as ~0.3 PAL in the Cambrian, more than PAL from Ordovician to Devonian, <0.1 PAL in the Permo–Triassic and fluctuating above and below PAL for the rest of the Phanerozoic. Estimates of oxygen levels in the Phanerozoic of Berkner and Marshall, and of Tappan, are shown in Fig. 3 together with base lines for carbon monoxide and methane combustion. (The limiting oxygen percentages for combustion of carbon monoxide and methane can be seen to correspond to ~0.3 PAL and 0.6 PAL respectively.) The carbon monoxide combustion threshold is necessarily the base line for natural burning, and therefore, charcoal production. Based on Tappan's estimate, charcoal could not have been produced from late Carboniferous to early Jurassic times. However, fusain bands and lenses are widely distributed in Northern Hemisphere Carboniferous bituminous coals[23], and in northern Europe there is abundant clastic fusinite in Rhaeto–Liassic and Middle Jurassic sediments[24–26]. Clearly, Tappan's estimates of oxygen levels based on phytoplankton production are incompatible with this evidence, while the Berkner–Marshall oxygen curve is above the carbon monoxide combustion threshold from the mid-Devonian onwards, and is, therefore, consistent with the geological record of fusinite. A different view is adopted by Cloud[27], whose estimate of oxygen levels is based on an assumption of steady accumulation of photosynthetic oxygen throughout geological time. Cloud argues that the Pasteur point (0.01 PAL) was attained early in the Earth's history (~2,000 Myr) and that levels of 0.1 PAL were reached in the late Precambrian. From his data the carbon monoxide combustion threshold would have been achieved by the late Cambrian, which is consistent with the geological record of fusinite.

The presence of the first charcoal in the geological record is an indication that oxygen levels in the atmosphere were above 0.3 PAL. The oldest sedimentary occurrences of charcoal largely in a non-carbonaceous matrix that we know of are in Lower Carboniferous strata from Donegal, Eire[28], and Fife,

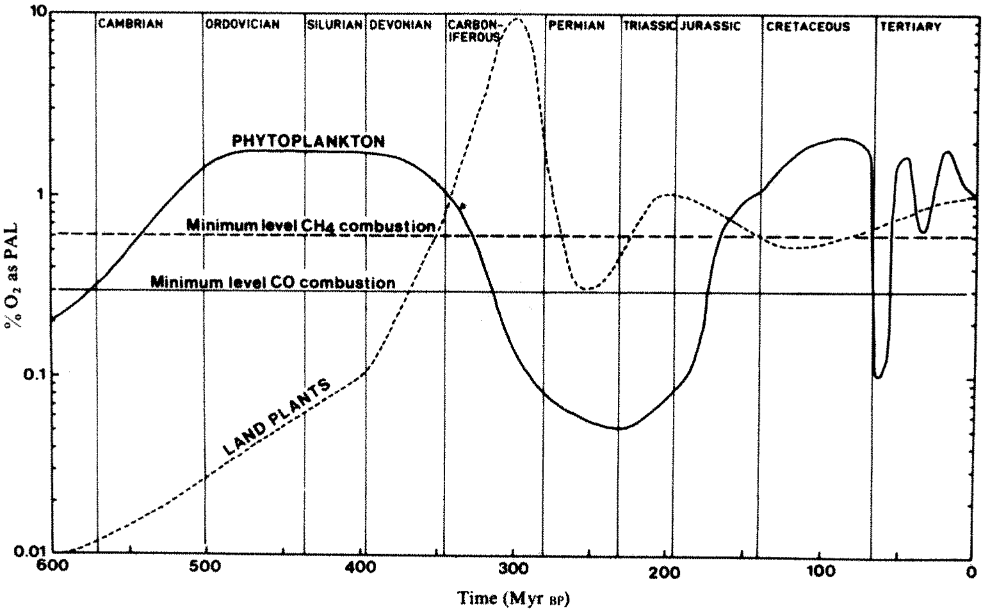

Fig. 3 Estimates of the level of oxygen in the atmosphere during Phanerozoic time based on the assumption that photosynthesis by phytoplankton was the main controlling factor[22] (solid line) and corresponding hypothetical levels assuming land plant photosynthesis to be the main controlling factor[21] (broken line). The estimate of Cloud[27] based on a steady build-up of oxygen level from all photosynthetic sources is not shown for reasons of clarity; this gives oxygen levels above 0.1 PAL at the start of the Phanerozoic and above 0.3 PAL by the late Cambrian, with a subsequent steady increase to PAL during the remainder of the Phanerozoic. The combustion thresholds for carbon monoxide and methane[19] which necessarily limit the occurrence of forest fire burning, are also shown.

Scotland. The oldest documented record of charcoal in a carbonaceous matrix is from a transitional Devonian–Carboniferous 'coal' from Pennsylvania[29]. In the present state of knowledge, it can therefore be argued that levels of oxygen greater than 0.3 PAL were attained at least by the latest Devonian and were subsequently maintained above this level. Thus the recognition of charcoal in sediments offers a useful quantitative indicator of the oxygen content of the atmosphere from the Devonian onwards.

Charcoal has a particular significance in another respect as a constituent of sedimentary rocks. Its inert character renders it highly resistant to microbiological or chemical degradation during sedimentation and subsequent diagenesis, while its thermal stability enables it to withstand high palaeotemperature of deep burial. It follows that from Devonian time onwards, charcoal could have made a significant contribution to the insoluble dispersed organic matter in sediments (kerogen). The total amount of kerogen is estimated to be some 500 times greater than all known coal deposits[30]. However, because of its dispersed nature, this vast reservoir of carbonaceous material is unlikely to be exploited despite the ever increasing need for new fuel sources. Natural burning produces air-borne carbon particles[31] and releases charcoal clasts at the site of fires[14]. Such inert carbonaceous products can contribute to kerogen. Air-borne carbon particulates are recognised in modern and Tertiary marine sediments[31,32], and structured fusinite micro-clasts occur in kerogen isolated from ancient sediments[33]. A study of these kerogen particulates in post-Devonian sediments may help to establish the geographical and temporal distribution of pyrolysis-derived components and thus reveal a record of past forest-fire activity.

Charcoal has been largely overlooked or ignored by both sedimentologists and palaeobotanists in sediments of all ages (except, perhaps, coals); in view of the geochemical and palaeoecological significance of its occurrence, attention could usefully be concentrated on the first appearance of charcoal in the geological record. We would be particularly interested to learn of any charcoal occurrences of Devonian or earlier age.

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Stable isotope and sea-level data from New Guinea supports Antarctic ice-surge theory of ice ages

Paul Aharon, John Chappell & William Compston

Institute of Advanced Studies,
Australian National University, Canberra, Australia

Two theories of glaciation have received considerable attention, the Milankovitch orbital theory[1,2] in which climatic change is ascribed to latitude-dependent variations in solar radiation that accompany changes in the Earth's orbital parameters, and the Antarctic surge hypothesis[3–6], in which a large Antarctic ice sheet 'surges' into the Southern Ocean thereby increasing the Earth's albedo, and the resultant cooling triggers the growth of ice in the Northern Hemisphere. The interval which encompasses the peaks of the last interglacial maximum at ~125 kyr (ref. 7) and its subsequent decline, with cooling of the North Atlantic[8] and rapid fall of sea level[9,10], presumably also includes the event that triggered the succeeding ice age. The raised coral reefs of Huon Peninsula, Papua New Guinea, contain a particularly good record of the interval 140–105 kyr (ref. 11), from which we present oxygen isotope and sea-level data which seem to require an Antarctic surge at 120 kyr, and which also have a bearing on the role of the Milankovitch factor.

The structure of Huon Peninsula reefs VIIa, VIIb and VI is shown in Fig. 1. Relative to the rising landmass, the essential sea-level changes represented by these reefs are [10,11]: (1) sea-level rise during building of reef VIIa; (2) minor fall during erosion at X in Fig. 1; (3) rise of >8 m during building of VIIb crest; (4) falling sea level, with continued growth of the patch-reef at Y during early stages; this regression eventually reached the base of reef VI; and (5) rise during building of reef VI. The envelope curve of sea-level changes interpreted from these and comparable reefs in Timor[9] is shown in Fig. 2*a*; the tectonic uplift factor has been subtracted[11–13]. Chronology is based on ^{230}Th/^{234}U determinations of these reefs[12,13], correlated with dated reefs in Timor where VIIa, VIIb equivalents are separated clearly[9]. Correlation extends to well dated reefs in Hawaii[14] which contains two units believed equivalent to VIIa, VIIb on the grounds that each reef-building phase essentially is controlled eustatically[9]. Pooling the ^{230}Th/^{234}U data puts VIIa at 133 ± 4 kyr and VIIb at 120 ± 3 kyr (ref. 9). Reef VI dates at 105 kyr and correlates with similar reefs in Timor[9] and Barbados[15]. The duration of building and emergence of VIIb are important in our subsequent discussion. First, between erosion unconformity X in Fig. 1 and the VIIb crest there is >8 m of

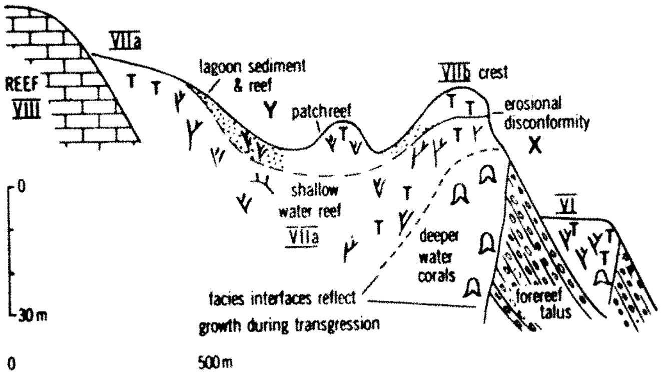

Fig. 1 Section of New Guinea reefs VIIa, VIIb, VI generalised from three separate sections (Gitua, Sazum, Sialum[11]). Sampling points shown by *Tridacna gigas* symbol (T).

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shallow water reef, representing 1–2 kyr of growth, on the basis of ^{14}C study of Holocene reef growth at the Huon Peninsula[16]. Second, between emergence of VIIb crest and cessation of coral lagoon–reef growth at points Y in Fig. 1, ~3 kyr minimum must have elapsed assuming both tectonic and glacio–eustatic factors acted at maximum known rates[10,11].

Stable isotope analyses from these reefs were based on the giant clam *Tridacna gigas*, for the following reasons. (1) Coral recrystallisation in the raised reefs is extensive and diagenetic exchange of carbon has been confirmed by ^{14}C analysis rendering corals unsuitable[17]. (2) Hermatypic corals fractionate stable isotopes in a manner related to growth habitat[18,19]. (3) The massive aragonite shell of *Tridacna gigas*, readily checked for diagenesis by microscope and X-ray diffraction methods, is shown by ^{14}C generally to remain immune from carbon exchange and diagenesis[17]. (4) Most importantly, our analyses of modern specimens indicate temperature-controlled isotopic equilibrium with surrounding seawater. Table 1 lists $\delta^{18}O$ for 23 determinations from seven modern specimens as -1.54 ± 0.19‰ PDB. The adjacent ocean yields -0.07‰ SMOW; using Epstein's[20] palaeotemperature equation plus 0.6‰ aragonite enrichment factor[21] gives an isotopic temperature of 27 °C, which agrees with measured sea-surface temperatures in the area.

Stable isotope data from each reef phase are given in Table 1, together with estimated sea-level and mean age data. Multiple samples (numbers as given) were collected in each reef zone and multiple determinations made of each. The procedure was as follows: to measure the environmental average it is necessary to sample the full growth-width of a *Tridacna*, as stable isotope analysis of single growth lamellae in modern specimens clearly shows season-dependent variation[22]. Accordingly, the massive hinge region was sliced radially and each slab scrutinised for algal borings and calcite veins. Flawed samples were rejected. A thin strip extending across each slab was crushed, X-rayed to ensure aragonite purity, and the powder roasted for 30 min at 400 °C to eliminate any possible organic shell material[20,23]. Extraction of CO_2 follows McCrea[24], and mass spectrometry was done on a modified MS-12 machine[25]. Instrument precision is ±0.05‰, reproducibility including chemistry is ±0.1‰.

Almost all samples grew in reef sites exposed to the open ocean, either in forereef or shallow reef crest positions. The only exceptions are those from the regression phase of VIIb (Table 1), which come from the patchreef and lagoon floor at Y in Fig. 1. Oxygen isotopes show significant variations across the interval 140–105 kyr, while the carbon isotopes do not. $\delta^{13}C$ shows no significant variation, first because its temperature dependency is three (ref. 26) to six (ref. 27) times smaller than for $\delta^{18}O$, and second because it has greater intrinsic variability related to local and diurnal $\delta^{13}C$ variations measured in the coastal waters[22].

Concerning the $\delta^{18}O$ variations, two factors are important for organisms in well mixed ocean waters. Temperature rise of 1 °C lowers $\delta^{18}O$ by about 0.24‰; glacio–eustatic sea-level rise of 10 m lowers $\delta^{18}O$ by ~0.1‰ due to dilution of oceanic oxygen by ^{18}O-poor ice meltwater[28]. These two factors are decoupled in Table 1 as follows. The difference between the modern *Tridacna* $\delta^{18}O$ value (-1.54‰) and that for a given reef phase is treated as if it were due to sea level being different from present, giving the $\delta^{18}O$ sea-level estimates of Table 1. Should this apparent sea level be significantly different, statistically speaking, from the sea level previously established for that reef phase then the 'unexplained' $\delta^{18}O$ residual is taken as being temperature related, for example, for reef crest VI (line 2, Table 1), $\delta^{18}O = -1.45$‰ difference from modern $= -0.09$‰, giving an apparent sea level of −9 m. This is not significantly different from the estimated sea level of −14 m.

In this way, only reef VIIb crest shows a $\delta^{18}O$ value which is not explicable in terms of sea-level change. It is 0.41‰ higher than modern; more importantly, it is 0.51‰ higher than the value for VIIa crest, which formed at a similar sea level 5–7 m higher than present (Table 1). This $\delta^{18}O$ difference is significant at better than 0.01 level (Student's *t*-test), and the difference lies between 0.40‰ and 0.62‰ at the 95% confidence level. As the sea level cannot account for this difference, we conclude that the ocean adjacent to Huon Peninsula was 1.7–2.5 °C cooler during VIIb crest building than for VIIa crest.

Cooling during VIIb crest building follows a sea-level rise of >8 m which commenced at least 1–2 kyr previously (discussed above). This agrees qualitatively with the Antarctic surge theory, which predicts both rapid sea-level rise and significant world cooling before onset of the northern glaciation. Note that the Milkankovitch factor cannot explain this cooling, as radiation 120 kyr ago was similar to the present (Fig. 2*c*), and in any case our *Tridacna* samples measure mean annual conditions, which do not vary significantly in Milankovitch terms.

We stress that our samples from reef VIIb formation, which indicate this cooling during the sea-level rise at 120 kyr, come from the seaward side of barrier reefs which were distant from river mouths and fully circulated by open sea. This 2 °C cooling poses a quantitative problem, if it reflects the arrival in the New Guinea region of cold water originating from an Antarctic surge. If all the heat for melting the floating ice came from the entire oceans (latent + sensible heat), net cooling would be only 0.3 °C. Either there is a more direct transfer via some component of the oceanic circulation, or global cooling arising from the extended iceshelf albedo is implicated. We note that the cooling effect seems largely to have disappeared in the tropical New Guinea

Table 1 Carbon and oxygen isotope data, sea levels and ages for New Guinea reefs of last interglacial episode

Reef phase*	Location†	Age estimate‡ (kyr)	Sea level (m)	No. of specimens	Determinations	$\delta^{13}C$ (PDB f)	$\delta^{18}O$ (PDB f)	$\delta^{18}O$ sea level (m)§	$\delta^{18}O$ residual (‰)‖	Temperature residual (°C)¶
Modern	S, G	0	0	7	23	2.25 ± 0.26	-1.54 ± 0.19	—	—	—
Reef VI										
crest	S, G	105	−14	2	8	2.73 ± 0.11	-1.45 ± 0.07	−9	NS	0
transgn	G	110	−40	1	3	2.76 ± 0.07	-1.00 ± 0.13	−54	NS	0
Reef VIIb										
regrn	S, G	11	−15	7	16	2.55 ± 0.25	-1.47 ± 0.14	−7	NS	0
crest	S, G	120	8	7	20	2.36 ± 0.28	-1.13 ± 0.09	−41	0.49	−2.0
Reef VIIa										
crest	S	133	5	8	16	2.47 ± 0.11	-1.64 ± 0.11	10	NS	0
transgn	Z	140	−40	1	3	2.32 ± 0.05	-1.24 ± 0.14	−30	NS	0
transgn	Z	144	−60	1	4	2.66 ± 0.09	-0.80 ± 0.16	−74	NS	0

* Reefs as in Fig. 1: transgn, transgressive shallow water facies; regrn, regressive facies.
† Locations according to profiles in ref. 11: S, Sialum; G, Gitua; Z, Sazum.
‡ Age estimates and sea levels according to refs.
§ $\delta^{18}O$ sea level $= (\delta^{18}O, \text{modern} - \delta^{18}O, \text{sample}) \times 10$ m.
‖ $\delta^{18}O$ residual $= (\text{estimated sea level} - \delta^{18}O \text{ sea level}) \times 0.1$‰.
¶ Temperature residual $= (\delta^{18}O \text{ residual}/0.24)$ °C. NS, not statistically significant.

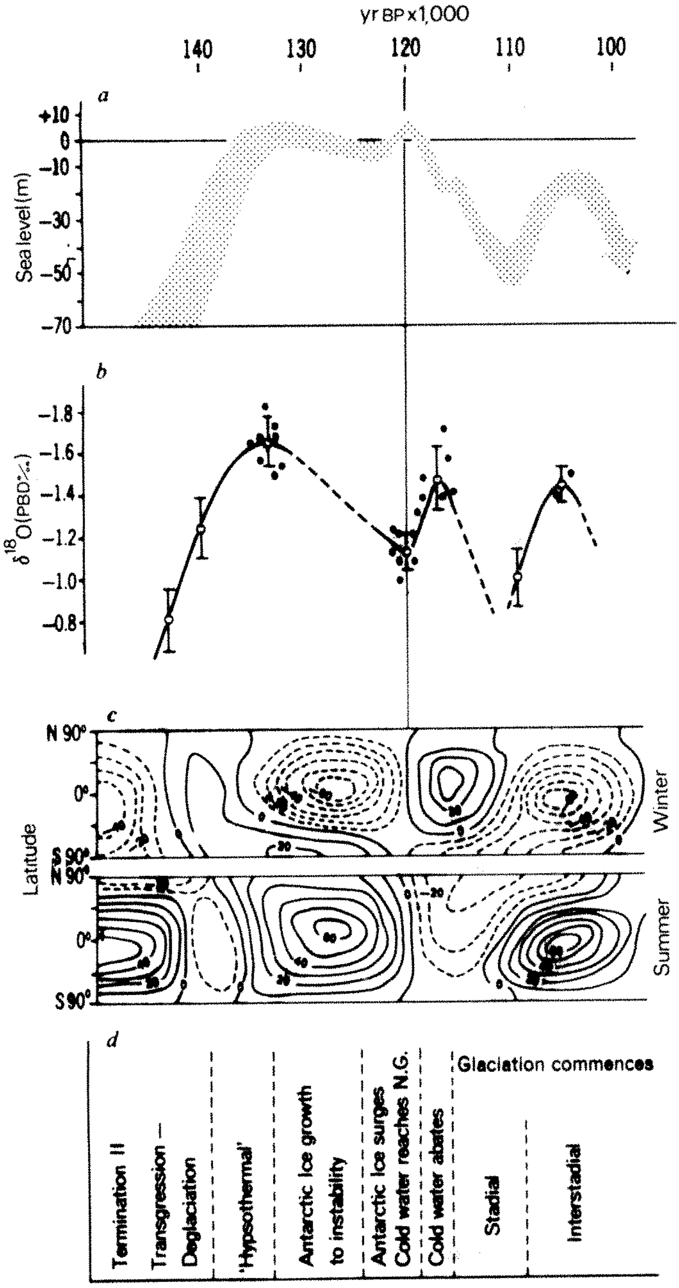

Fig. 2 Sea levels, $\delta^{18}O$, and insolation changes, 150–100 kyr BP. *a*, Sea levels[10,11]; *b*, $\delta^{18}O$ from *Tridacna gigas*, New Guinea reefs. ●, Specimen values; ○ are group means for each stratigraphic unit, bars are group 1σ values. *c*, Deviations of solar radiation from present values in Langleys per day[30]. *d*, Interpretation.

about 3,000 yr after the VIIb peak, that is, when the VIIb patch reefs formed (point Y in Fig. 1), as temperatures similar to present are indicated (Table 1). (Although this result is from samples which grew within, as against outside, a lagoon, we believe lagoon ponding was not involved as the geometries are similar to modern, fully flushed lagoons at Huon Peninsula.) Hence, irrespective of the mechanism which produced the 2 °C cooling during the VIIb sea-level rise, it seems that its manifestation waned in the tropics while the northern glaciation was initiated. We do not know whether the surging ice mass was more likely to have been East Antarctica (Wilson hypothesis[3,4]) or West Antarctica (Mercer hypothesis[5,6]).

Note also that the Milankovitch factor predicts strong warm-summer–cool-winter contrast during the 125–130 kyr interval, a condition normally taken as inimical to northern continental ice growth. Such a radiation pattern may favour enhanced summer snowfall in Antarctica, leading to icesheet building towards instability. This might explain the minor sea-level decline from the VIIa peak before the VIIb rise. Figure 2*d* summarises the sequence of events, and we conclude that an Antarctic ice surge seems likely to have initiated the last glaciation.

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Isostatic equilibrium grounding line between the West Antarctic inland ice sheet and the Ross Ice Shelf

Larry L. Greischar & Charles R. Bentley

Geophysical and Polar Research Center, University of Wisconsin-Madison, Madison, Wisconsin 53706

The Ross Ice Shelf is widely believed to be a Holocene feature, that is, during the late Pleistocene the inland (grounded) ice sheet extended out nearly to the continental shelf break in the Ross Sea, and retreated to its present position between 5,000 and 10,000 yr BP. If so, and if the grounded ice sheet remained in that position for at least 10,000 yr, then a sea floor uplift of the order of 100 m is still to be expected in the grid western part of the Ross Ice Shelf. Even for a smaller and more ephemeral extension of the grounded ice, the uplift would still be several tens of metres. Recently reported measurements indicate that there are extensive areas near the present grounding line where the water layer beneath the ice shelf is thin enough so that uplift would lead to a grounding line advance of at least 100 km. As the sea floor depth generally increases towards the grid east, however, the maximum extent would not be past the middle of the present Ross Ice Shelf.

Seismic soundings of submarine topography and radar soundings of ice thicknesses were made at sites on a 55 km grid covering the Ross Ice Shelf, Antarctica, during the Ross Ice Shelf Geophysical and Glaciological Survey (RIGGS)[1–5]. The submarine topography, characterised by a general deepening to

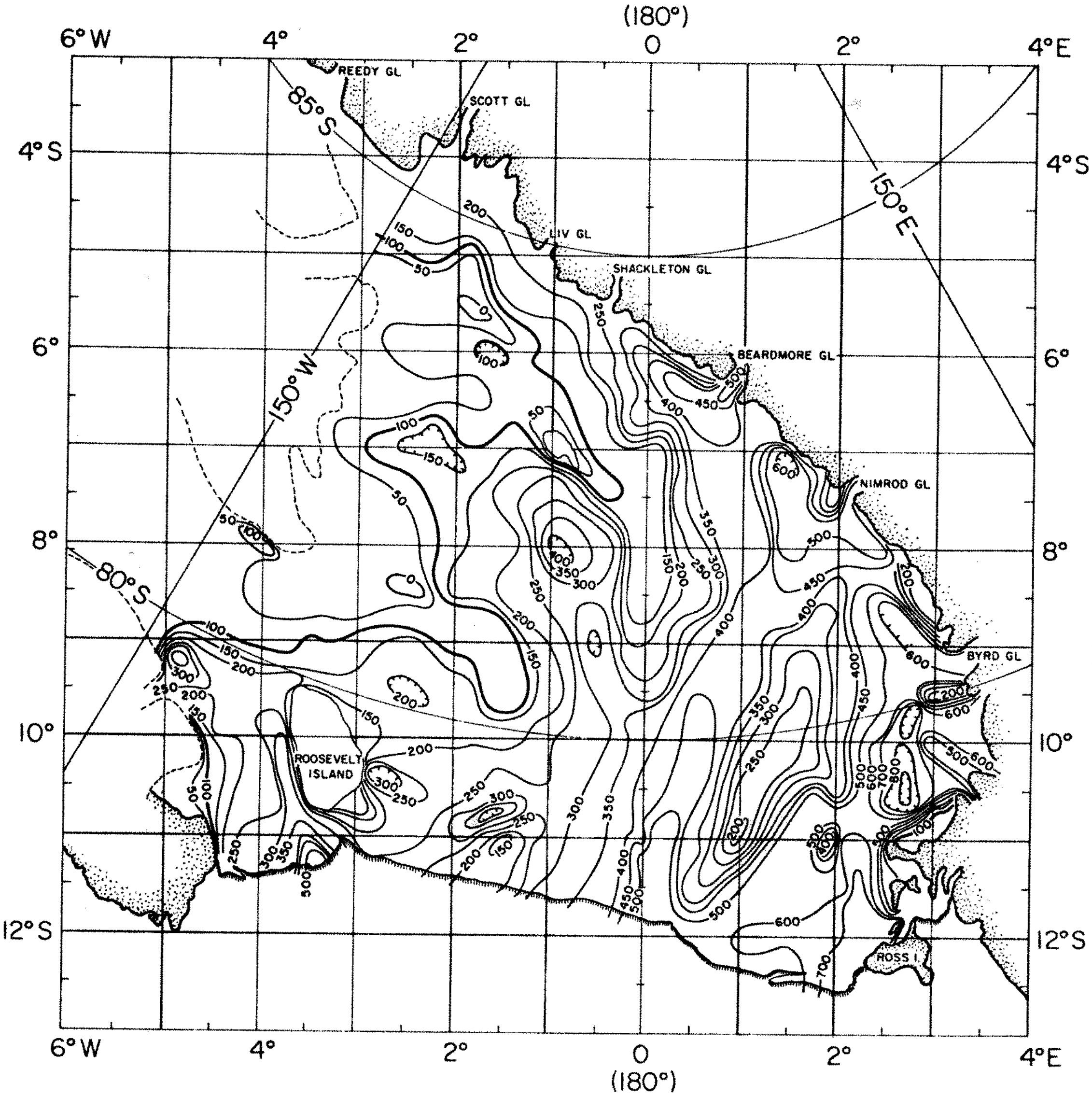

Fig. 1 Water depth beneath the Ross Ice Shelf. Standard grid coordinates are shown; grid north is towards Greenwich, and grid meridians are parallel to 180° W longitude. The present grounding line between the Ross Ice Shelf and West Antarctica is shown by the dashed line which is discontinuous in areas where ice streams enter the ice shelf. The contour interval is 50 m for depths from 0 to 500 m, and 100 m for greater depths.

the grid eastwards on which is superimposed a curving ridge–trough system, is reflected in a contour map of the 'water depth' (defined as the thickness of the water layer beneath the ice shelf) (Fig. 1). The position of the primary grounding line between the Ross Ice Shelf and West Antarctica was determined from airborne radar sounding measurements of ice surface elevations and strength and polarisation of bottom reflections[6]; the latter indicate whether water or rock is present directly below the ice. Seismic, radar and altimeter measurements made on the surface[1–3] were used for control. The surface topography shows the grounding line to be deeply indented beneath the West Antarctic ice streams, although scattering of the radar signals by heavy crevassing prevents accurate determination of its position. The ice shelf is also grounded at Roosevelt Island (grid position 10.0° S, 3° W), Crary Ice Rise (7.0° S, 1.0° W), near the Steershead Crevasses (8.5° S, 2.5° W), and possibly at RIGGS station F7 (5.5° S, 1.8° W) (see Fig. 3). Beneath much of the grid western part of the ice shelf, the water depth is < 100 m, and in some places it is only a few tens of metres.

Stuiver *et al.*[7] have reconstructed the Antarctic Ice Sheet at the 18,000 yr BP glacial maximum. Their reconstruction indicates that West Antarctic ice was then ~2,500 m thick near the present grounding line, and that the margin coincided with a line of sills parallel to the edge of the Ross Sea continental shelf. Two-by-two-degree mean isostatic gravity values at RIGGS stations on the Ross Ice Shelf[8], which are as much as 15 mGal negative compared with a fourth-order satellite gravity field[9], strongly suggest that glacio–isostatic depression of the grid western part of the present Ross Ice Shelf area still remains. Recent evidence from Ross Sea cores also suggests that grounded ice reached the continental shelf margin during the late Pleistocene[10]. Thomas and Bentley[11] calculated that if such a Ross Ice Sheet existed 18,000 yr ago, then it probably retreated rapidly to its present position between 13,000 and 7,000 yr BP. Geological data in the McMurdo Sound and dry valley areas yield minimum ages of ice retreat ranging from 3,000 to 10,000 yr BP (ref. 12) and have further been interpreted as showing that the grounding of the ice sheet occurred more than 47,000 yr ago[13].

Although some recent analyses of marine and glacial geological evidence indicate that only minor expansions of the grounded ice occurred during the Quaternary[14–16], we believe that the preponderance of evidence favours the existence of an extended grounded ice sheet of some form. The following analysis is based on the initial working hypothesis that a grounded 'Ross Ice Sheet' existed long enough to become isostatically compensated (~10,000 yr), and that it was removed rapidly between 5,000 and 10,000 yr BP. In that case, isostatic uplift of the loaded area should still be occurring. The grounding line between the West Antarctic Ice Sheet and the Ross Ice Shelf will advance as the sea floor rises. We have estimated the uplift remaining in the grid western portion of the Ross Ice Shelf, assuming a former ice thickness of 2,500 m (ref. 7). Following Bennett[17], we take unloading to have been instantaneous, and assume a simple exponential model of uplift:

$$Z = Z_0 \exp[-(t-t_0)/t_r] \quad (1)$$

where Z is the amount of uplift remaining, Z_0 is the original isostatic depression due to the ice load, t_0 is the time of load removal and t_r is the time constant of isostatic rebound. Z_0 can be estimated from the former ice thickness, T, and the estimated average depth of the present sea floor, $\bar{D}$, by

$$Z_0 = \frac{\rho_i}{\rho_m} T - \frac{\rho_w}{\rho_m}(\bar{D} - Z) \quad (2)$$

where ρ_i, ρ_w and ρ_m are densities of ice, sea water and mantle rock, respectively. Substitution of equation (2) into equation (1) yields an expression for the remaining uplift:

$$Z_p = \frac{\left[\frac{\rho_i}{\rho_m} T - \frac{\rho_w}{\rho_m} \bar{D}\right] \exp[-(t_p - t_0)/t_r]}{1 - \frac{\rho_w}{\rho_m} \exp[-(t_p - t_0)/t_r]} \quad (3)$$

wherein Z_p and t_p are the present remaining uplift and the present time.

The average water depth at RIGGS stations, $\bar{D}$, is ~650 m. The relaxation time constant, t_r, was estimated for an area the size of the Ross Ice Shelf from values measured in the Northern Hemisphere and predicted by model studies (Fig. 2, adapted from Cathles[18]). The linear dimension of the Ross Ice Shelf is of the order of 1,000 km, so t_r was chosen to be ~4,400 yr, the value used by Cathles for central Fennoscandia. Note that t_r is relatively independent of both the linear dimension of the ice load and the crustal rigidity for ice loads of this size. Densities used in equation (3) were $\rho_i = 0.91$ Mg m^{-3}, $\rho_w = 1.03$ Mg m^{-3}, and $\rho_m = 3.33$ Mg m^{-3}.

The results of the calculation (Table 1) show a remaining uplift ranging from 50 to 170 m. This estimate agrees well with that from the mean isostatic gravity anomalies which suggest as much as 150 m residual depression near the grounding line[8]. Because of the linearity of equation (1), these figures can easily be modified to fit other assumptions. Thus, for example, if the initial depression were only half as large as has been assumed, the uplift remaining would still be at least 50 m if the time since removal is less than 7,000 yr. That initial depression, in turn, could correspond to a fully compensated grounded ice sheet whose margin extended only about as far as a line extending from Edward VII Peninsula to Byrd Glacier, to a fully extended ice sheet which was in position for only ~3,000 yr, or to a 'low profile' extended ice sheet that might be compatible with the shape of internal reflecting horizons found in the West Antarctic interior[27]. Thus 50 m is a reasonable approximation to an uplift to be expected even assuming a smaller or more ephemeral ice sheet.

Table 1 Uplift remaining since ice removal

Time since ice removal (yr)	Remaining uplift (m)
5,000	170
6,000	135
7,000	105
8,000	80
9,000	65
10,000	50

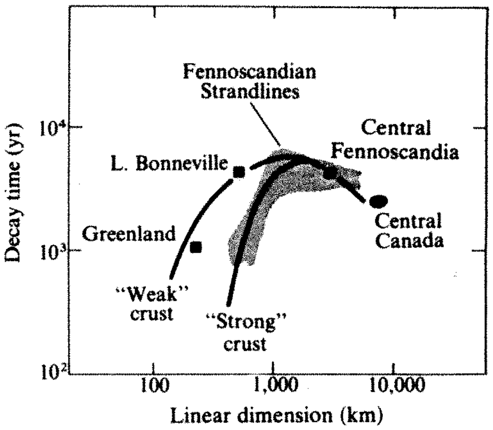

Fig. 2 Decay time constant for isostatic uplift versus the linear dimension of areas formerly covered by ice sheets (Canada, Greenland, Fennoscandia) or water (Lake Bonneville). Adapted from Cathles[18].

We have, therefore, estimated a maximum and minimum predicted advance of the grounding line based on a remaining uplift of 50–170 m and the assumption that the ice shelf thickness remains constant at the grounding line (Fig. 3). The maximum predicted grounding line extends grid northeastwards across the ice shelf from the seaward end of Roosevelt Island, and is deeply indented by extensions of the present ice streams. The minimum predicted grounding line advance would result from an advance of the central part of the present grounding line, with additional minor advances occurring near Edward VII Peninsula, Roosevelt Island and Crary Ice Rise.

The present rate of uplift is, from equation (1):

$$\left.\frac{dZ}{dt}\right|_{t=t_p} = -\frac{Z_p}{t_r} \quad (4)$$

for $Z_p = 110$ m, the uplift rate is 25 mm yr^{-1}. Division by the water depth gradient, which varies from 10^{-4} to 10^{-3} near the present grounding line, yields a rate of advance of the grounding line of 25–250 m yr^{-1}. At this rate, several thousand years would be required for the grounding line to advance to its predicted maximum position. As uplift occurs, however, points seaward of the primary grounding line will be pinned on high points of the sea bed, forming new ice rises. Compressive strains upstream from these pinning points will greatly accelerate the rate of grounding line advance. We believe this is now occurring upstream of Crary Ice Rise (grid 7.0° S, 1.0° W)[19], and also near the Steershead Crevasses (grid 8.5° S, 2.5° W).

The possibility that sedimentation may have been an important factor during isostatic adjustment can be dismissed on the basis of ocean floor cores taken at station J9 (grid 7.5° S, 1.5° W). Little or no Pleistocene or Recent sediment is present at that location[20], whereas a sedimentation rate of the order of 55 mm yr^{-1} would be required to produce a present state of isostatic equilibrium[17]. Furthermore, even if the basal layers of the ice shelf contain as much as 10% morainal material, the latter sedimentation rate would imply a bottom melt rate of ~0.5 m yr^{-1}, whereas temperature and resistivity measurements indicate a near-zero melt-freeze rate at J9 (ref. 21). Also, preliminary observations on ice cores from the lowest layers, obtained at J9 in December 1978, suggest an average bottom freezing rate of ~0.03 m yr^{-1} (J. W. Clough and I. A. Zotikov, personal communication).

Peltier[22] has pointed out that the longest wavelength components of the depression may 'feel' the Earth's core, and decay non-exponentially. On any realistic Earth model, however, that applies only to wavelengths of many thousands of

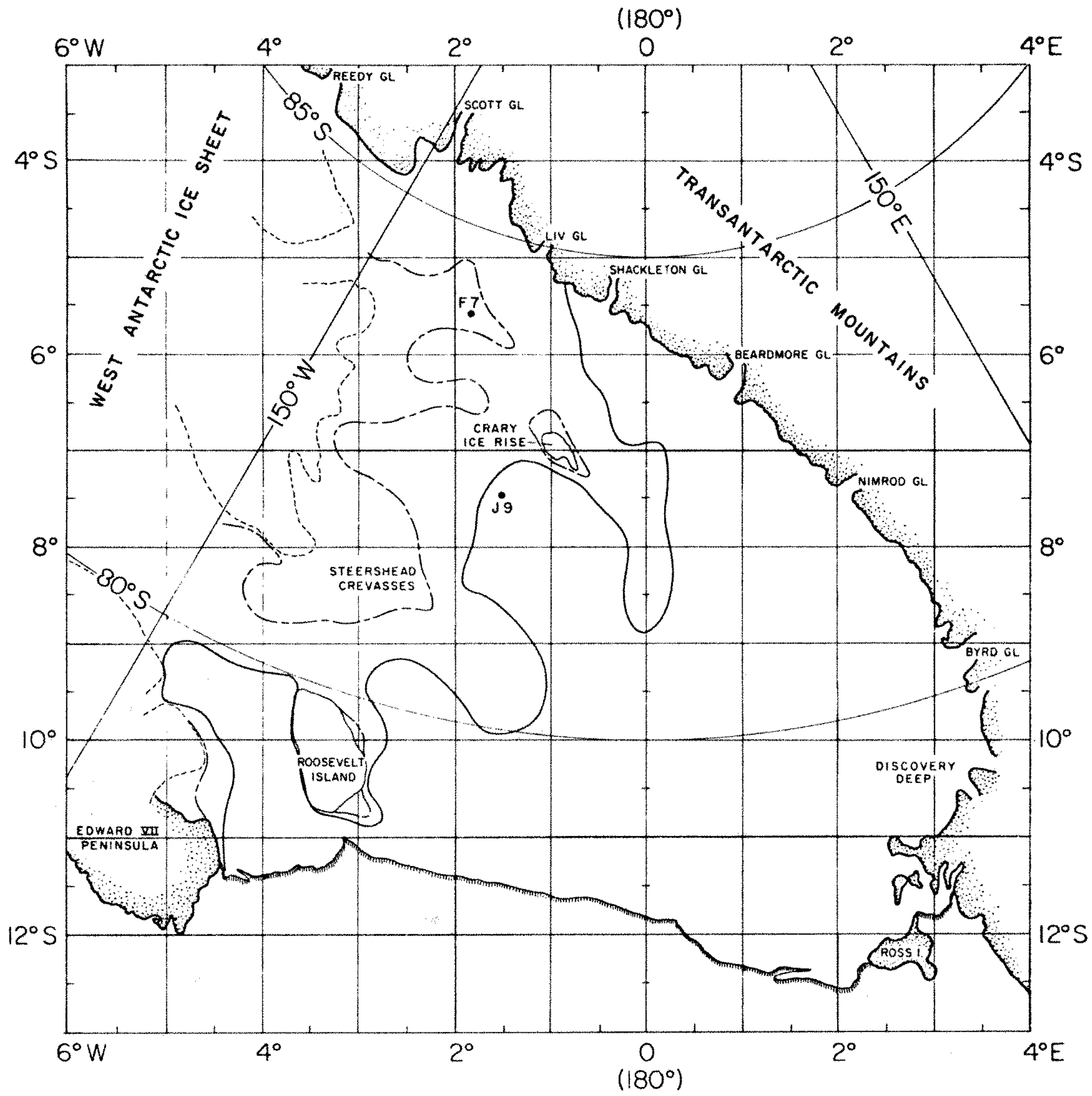

Fig. 3 Minimum (dashed line) and maximum (solid line) predicted seaward advance of grounding line, based on 50–170 m of additional isostatic uplift.

kilometres—much larger than the Ross Ice Shelf. Using Peltier's[22] Green functions in the Farrell–Clark[23] model and the Stuiver *et al.*[7] Antarctic reconstruction, Lingle and Clark[24] have computed a relative sea-level curve for 2,000 yr BP to the present near the centre of the Ross Ice Shelf. As their curve indicates a rate of emergence (~11 mm yr^{-1}) that is nearly constant rather than exponentially decreasing with time, it is impossible to use their method at the current stage of development to estimate the remaining uplift. Furthermore, as exponential decay describes well the uplift data from central Canada[25,26], it seems to be a reasonable assumption for uplift of the sea floor beneath the Ross Ice Shelf.

The model presented here does not, of course, consider the possibility of non-equilibrium of the West Antarctic ice sheet. Growth or shrinkage of that ice sheet due to climatic or inherent dynamic causes could produce grounding line movement which is large compared with that from isostatic uplift. However, if the West Antarctic inland ice sheet is approximately in steady state, an advance into the Ross Ice Shelf is still to be expected. The corresponding lowering of world sea level would be of the order of 0.5 m.

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Widespread episodes of stream erosion during the Holocene and their climatic cause

G. Robert Brakenridge

Department of Geosciences, University of Arizona, Tucson, Arizona 85721

Quaternary fill-terrace sediments along a 6-km reach of the Pomme de Terre River in south-central Missouri (38°06′ N, 93°26′ W) have yielded abundant vertebrate fossils, pollen, and archaeological remains[1]. Numerous radiocarbon dates have also been obtained. When these are combined with stratigraphic results (described by Haynes and myself), an 'alluvial chronology' of floodplain depositional history can be constructed. The Holocene portion of this chronology is summarised here and compared with other dated alluvial and proxy-climatic sequences from the northern mid-latitudes. From such a comparison, considerable insight may be gained into the operation of synoptic climatological controls over fluvial sedimentation and erosion.

The state of our current knowledge on the floodplain history of the Pomme de Terre River is given in Fig. 1 which shows a plot, on an *x*–*y* coordinate system, of the sample elevations above local river level of 21 radiocarbon dates, and also the measured elevations of the tops and bottoms of each mapped stratigraphic unit. Full details regarding local stratigraphy exposed along this valley by backhoe-trenching are given elsewhere[2,3].

Periods of slow or fast floodplain aggradation, stability and erosion during the past 11,000 yr are immediately recognisable on Fig. 1. One facet of river history made especially evident is that conditions of 'grade' or stability have prevailed for only limited periods of time during the Holocene. Instead: (1) a major aggradation occurred between 10,500 and 8,100 yr BP, coevally with loess deposition on local hilltops[2]; (2) following a brief erosional event between 8,100 and 7,500 yr BP, the river rapidly readjusted its grade; (3) floodplain stability prevailed from 7,500 to ~5,000 yr BP; (4) by 4,600 yr BP a major change in long-term stream regime had occurred; and (5) since this time, rapid aggradation has alternated with closely-spaced intervals of erosion. This last period, characterised by lack of long-term stability, continues to the present.

The complexity of this Holocene alluvial sequence is of special significance to workers who map river terraces on the basis of their relative elevations above river level. The lowest well defined terrace by the Pomme de Terre River is the 'T1' level, about 4 m above the modern floodplain (T0). However, a pronounced break in slope between this terrace level and T0 is visible at only a few locations in the valley, where fortuitous erosion has removed intervening fills at intermediate levels between T1b2 and T0 (Fig. 1). Given this situation, some alluvial terraces along other rivers may well signify primarily that portions of the local depositional record are missing.

Comparison of the Pomme de Terre's floodplain history with regional long-term climatic trends, as evidenced by pollen-derived vegetational history, suggests a correlation between the two. The 7,500–5,000 yr BP period of floodplain stability along this river occurred during the 8,700–5,000 yr BP 'Prairie Maximum' in Missouri[4]. This former plant geography is believed to indicate a climate characterised by less effective precipitation, because the Prairie/Deciduous Hardwood Forest boundary was located far to the east of its modern location. Following the return of more moist regional climates ~5,000 yr BP, long-term stream regime changed. Closely spaced episodes of stream erosion and deposition began, and have continued up to the present.

What is the controlling factor causing long-term floodplain stability or instability along this stream? The timing of mid-Holocene stability along the Pomme de Terre is similar to the time of stability concluded by Haynes for many western US streams: 7,100–5,700 yr BP (ref. 5). This tends to dismiss Prairie Maximum grassland vegetation itself as the primary cause of floodplain stability: no such vegetation has been inferred for the mid-Holocene in the now semi-arid western US stream basins. A better hypothesis is that stability in both Missouri and the western US was the direct result of greatly increased recurrence intervals for moderately sized floods, due to pronounced zonal upper atmospheric circulation patterns at this time[6,7].

Comparing the Pomme de Terre sequence with others in the northern mid-latitudes tests a corollary to this hypothesis: that climatic periods of very high meridionality in upper atmospheric circulation should be accompanied by periods of instability along streams[7]. Horizontal bars extending temporally to the oldest and youngest date from each Pomme de Terre depositional unit are shown in Fig. 1. Gaps between these bars are the time intervals bracketing fluvial erosion at this site. Following this same method, two other published alluvial sequences were plotted adjacent to the Missouri chronology on Fig. 2. Haynes[5] produced the western US chronology by compiling radiocarbon dates from lithostratigraphic units mapped over a large portion of the western US. He described the considerable dendrochronological and archaeological evidence in the western US for an erosional interval some time between 750 and 450 yr BP, although this event was not shown by the few radiocarbon dates then available for this period. Recent ^{14}C dating of alluvium in northern Arizona confirms regional erosion ~450 yr BP (W. Robinson, personal communication) and this event is therefore also indicated on Fig. 2 for comparison. The central European chronology is my compilation of results presented by others[8–11]. It is based partly on dates obtained by dendrochronological analysis of fossil tree stumps and logs found in alluvial deposits. Because such dates are in calendar and not radiocarbon years BP, every such tree-ring-based date from these studies was first converted into radiocarbon years BP using a standard calibration curve[12] before plotting on Fig. 2.

The three alluvial chronologies thus compared show four coincident or overlapping time intervals bracketing fluvial erosion (black bars on Fig. 2). Stream erosion at 5,000–4,900, 2,900–2,500, 1,600–1,500 and 400–500 radiocarbon yr BP is compatible with all three alluvial sequences.

Other recent alluvial chronological work supports the existence of very widespread Holocene erosional episodes at these times. In southwestern Wisconsin, Knox and his students have demonstrated regional stream erosion between 6,000 and 8,000 yr BP, before 4,400 yr BP, before 3,100 yr BP and

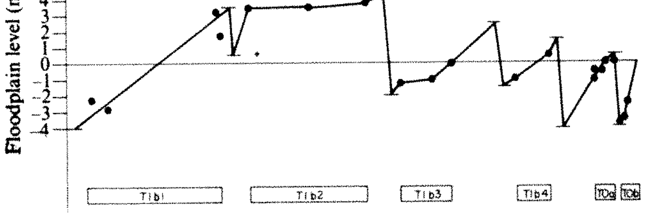

Fig. 1 Holocene level history of the Pomme de Terre River, southern Missouri, US. ●, Alluvial radiocarbon dates; + shows one of these dates that has poor reliability due to a large laboratory standard deviation. Short horizontal lines are undated stratigraphic tops or bottoms of each alluvial unit. Elevations are in metres above or below the modern floodplain, set at zero. Laboratory numbers of dates shown on this diagram are: ISGS-35, 48; SMU-429, 430, 431, 485, 500; UCR-819, 820, 823, 824; M-1900, 2281, 2333; GAK-1170, 1172; TX-1411, 1454, 1466; and A-867.

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between 1,800 and 1,225 yr BP (ref. 13). At the Koster site in southern Illinois, Butzer infers stream erosion at 5,000, 2,000 and 1,200 yr BP (ref. 14), although the latter two episodes are "fixed in relation to the regional archeological sequence" (personal communication). In Austria, along the Danube River, Fink's dates and cross-sections[15] support erosion between 8,500 and 7,000 yr BP, shortly before 4,200 yr BP, before 2,390 yr BP, shortly before 1,700 yr BP and between 560 and 400 yr BP. Finally, in Poland, Jersak dates an erosional unconformity in alluvial sediments between 8,620 and 6,160 yr BP[16], and Lindner presents a cross-section showing the basal portion of an alluvial fill dated at 1,850 and 2,060 yr BP (ref. 17).

Together, this is convincing evidence that sporadic episodes of widespread stream erosion have occurred during the past 5,000 yrs in the northern mid-latitudes. This result does not contradict dis-synchroneity of alluvial sequences at other times. Thus, stream erosion between 8,500 and 6,000 yr BP occurred at most localities mentioned above, but the data so far available are compatible with either: two closely spaced erosional intervals centering on 7,700 and 6,500 yr BP, and perhaps felt only locally, or one time-transgressive event between 8,000 and 6,500 yr BP, beginning earlier in eastern Europe and central North America, and later in central Europe and western North America.

The cause of the widespread stream erosion at the times shown in Fig. 2 is suggested by the timing of the Northern Hemisphere 'little ice ages'. These chronologies of mountain glacier advances have been assembled by Quaternary geologists working largely independently. However, their work shown in Fig. 2 demonstrates that Holocene glacial activity has been clustered around certain time intervals, even when the most divergent record (no. 6 in Fig. 2) is included. Widespread fluvial erosion seems to occur approximately at the beginning of little ice age climatic phases.

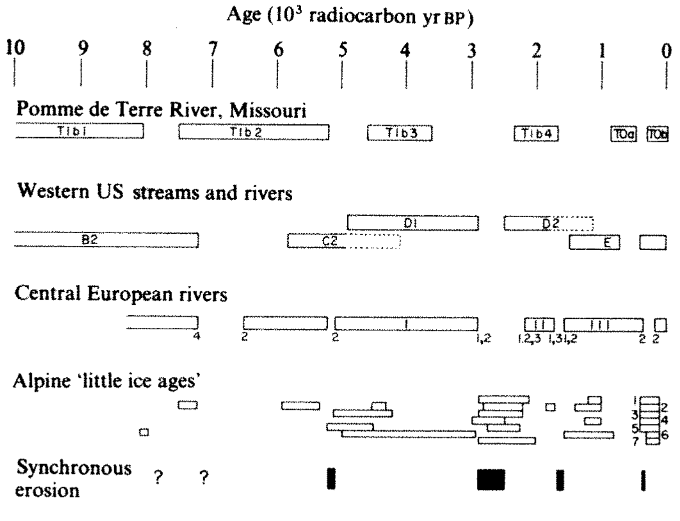

Fig. 2 Holocene alluvial and glacial chronologies compared. Key numbers 1–4 for 'Central European Rivers' refer to refs 8–11, respectively. Key numbers for 'Alpine Little Ice Ages' indicate the following references: 1, Alaska, ref. 21; 2, Swedish Lapland, ref. 21; 3, British Columbia and northern Washington, ref. 22; 4, Norway, ref. 23; 5, glaciations common to the eastern and western Alps, ref. 24; 6, Colorado Rocky Mountains, ref. 25; 7, Mexican volcanoes, ref. 26.

Other proxy palaeoclimatic indicators support climatic change occurring at these times, and help to clarify the nature of these changes. According to the California tree-ring results of LaMarche from both upper and lower treeline[18], the times of widespread glacial advances and stream erosion were characterised by rapidly decreasing land temperatures and increasing precipitation. Unfortunately, there are no such very long tree-ring climate series from the central US or Europe, and most pollen data from the central US are not sensitive enough to record short-lived climatic changes. However, "reconstructions of late-Holocene positions of treeline in Keewatin and Mackenzie, NWT, Canada, indicate that treeline was located south of its present position at about 2,900, 1,800 and 800 radiocarbon yr BP" and this is compatible with "increases in the strength of the meridional component of the large scale upper atmospheric circulation" at these times[7]. Moreover, Frenzel concludes that a variety of palaeoclimatic indicators from southwestern Europe also support greater meridionality at 5,200–4,400, 3,500–3,000, 1,700–1,200, and 800–600 yr BP, with shorter cooler and/or wetter phases ~2,700–2,500 yr BP, and within the past 600 yr[6]. Certainly it is hazardous to attempt detailed climatic comparisons over large areas on the basis of diverse palaeoclimatic indicators. Yet certain large scale changes have apparently left relatively unambiguous traces. If climatic change was the ultimate cause for recurring episodes of stream erosion during the past 5,000 yr, then the assembled palaeoclimatic evidence favours intensified meridionality in upper atmospheric circulation as the direct cause. This hypothesis was first advanced by Knox[7], on the basis of a statistical analysis of alluvial radiocarbon dates.

Because river channels are formed by floods with a recurrence interval of between 1 and 2 yr[19], channel widening or vertical channel incisement, therefore, occurs whenever floods of such recurrence intervals increase in size. During modern times, significant increases in meridional upper atmospheric circulation have been shown to result in such flooding over large areas[20]. Therefore, widespread episodes of Holocene stream erosion are to be expected if climatic regimes characterised by highly meridional circulation occurred in the past. Proxy palaeoclimatic records suggest that such regimes did occur, and at the correct times. Positive glacial mass balances in several mountain ranges resulted, even as increased flooding and discharge occurred along lowland streams. In contrast, one long interval of predominantly zonal flow was characterised by floodplain stability.

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Age of volcanism and rifting in southwestern Ethiopia

A. Davidson

Geological Survey of Canada, Ottawa, Canada K1A OE4

D. C. Rex

University of Leeds, Leeds LS2 9JT, UK

It has been suggested that volcanism in the Ethiopian region of the Afro-Arabian Rift System has migrated with time, both laterally towards the present axial zone[1-3] and longitudinally southwards from the Red Sea[4,5]. Field data and K-Ar isotopic ages from southwestern Ethiopia, summarised below, indicate that volcanism in this area began earlier than previously suspected, and that Quaternary volcanism was not restricted to the axial zone. That major rifting in this region did not happen until the late Miocene[6,7] is corroborated.

The Afro-Arabian Rift System in southwestern Ethiopia is particularly wide and complex[8], embracing from east to west the southern termination of the Main Ethiopian Rift (Ganjuli graben[9,10]), the Stefanie Rift, and three northward-terminating branches (Usno, Omo and Kibish) of the Turkana (Lake Rudolf) Rift. Rugged terrain between the Ganjuli graben and the western edge of the Ethiopian Highland, 450 km north-west, exposes crystalline basement overlain unconformably by various Tertiary and Quaternary volcanic rocks[11,12] (Fig. 1).

The Tertiary volcanic rocks are broadly divisible into two categories, based on whether erupted before rifting or not. Among those in the pre-rift category, some relative age distinctions can be made based on local unconformities within flow successions or on the nature of the unconformity on crystalline basement. The oldest suite, referred to as the main sequence, overlies crystalline basement with a characteristic, thin, basal unit of red, indurated, residual grit. Continuity of this uniform grit, not faulted or eroded before covering by lava, coupled with lack of both organic remains and evidence of fluviatile origin, suggest that most of this region was a featureless, dry peneplain, probably at low elevation, at the inception of Tertiary volcanism. Younger flows, generally conformable on and thus difficult to separate from the main sequence in the central north-east part of the area, overstep the main sequence on to basement to the west, south-west and south, and all lack a basal, red grit. Phonolite flows and small intrusions respectively overlie and cut all earlier flows and comprise the youngest pre-rift igneous rocks.

In the post-rift category, flood basalt sheets lie with marked unconformity on tilted pre-rift flows and are channelled by rift-related topography. Quaternary volcanism not only occurred locally within the Stefanie and Turkana Rifts but also on adjacent highland blocks, notably in the north-west where it built a huge shield astride the Tertiary unconformity, far removed from the axial rift zone.

Conventional K-Ar isotopic ages were obtained by analytical procedures described by Briden *et al.*[13] for basalt samples collected throughout the area from the base of successions that lie on crystalline rocks. Ages for rhyolite within the main sequence and for two phonolite intrusions were also determined. Age data are given in Table 1; sample locations are plotted in Fig. 1. Of 12 samples from the base of the main sequence, five cluster at ~40 Myr, three from near the Sudan border are distinctly older at ~48 Myr, one (C774) is anomalously old, and three are significantly younger. Of the latter, K095 is judged anomalous because it lies several hundred metres below rhyolite (A515) dated at 32.7±1.3 Myr. Two periods of extensive younger basalt effusion are documented by clusters of ages at ~32 Myr (Gok and Makonnen basalts in the north-west, Fejej basalt east of Lake Turkana) and ~20 Myr (Teltele basalt in the south-east and Surma basalt near the Sudan border). Ages of C775, 23.1 Myr, and B425, 54.7 Myr, are unexplained anomalies at unconformities where other age data are consistent. Phonolite in the Stefanie Rift is dated at ~13 Myr. Basalt (4.2 Myr) in outliers east of Lake Turkana is much younger than the Fejej basalt which it oversteps.

The age data accord well with published ages from the same region. Early main sequence flows are of the age suggested for the pre-Oligocene Ashangi basalts of central Ethiopia[2,10]. Makonnen, Gok and Fejej basalts are similar in age to the Aiba basalts of central Ethiopia[1,2,15,16] and to early basalts in northern Kenya[17]. 35–37 Myr ages obtained from low in the main sequence near the south end of the Main Ethiopian Rift are midway between these two groups, and 31 Myr old flows occur higher in the same succession without an unconformity between them[9,10]. The much younger Teltele and Surma basalts have probable age equivalents in flows overlying Fejej basalt east of Lake Turkana[18], and in the middle part of the pre-rift succession in the lower Omo Valley[19]. Phonolite igneous activity is the same age as in adjacent Kenya[17,20]. The post-rift basalt north-east of Lake Turkana correlates closely in age with the Mursi basalt in the Omo Valley[7,19], and with the Harr basalt east of Lake Turkana (R. T. Watkins, personal communication).

Table 1 Sample data

Location, map-unit, and position in section	No. in Fig. 1	Sample no.	%K	Volume radiogenic ^{40}Ar (std. cm^{3}g^{-1} × 10^{-6})	% radiogenic ^{40}Ar	Age (Myr)
Main sequence						
Sudan–Ethiopia border; analcime basalt flows on crystalline basement. Basal red grit not observed	1	A484	0.599	1.0989	19.5	46.6±2.0
	2	B608	0.437	0.8349	32.1	48.5±1.9
	3	I045	0.427	0.8312	41.5	49.4±1.9
Basal flood basalt flows, unconformable on crystalline basement with basal grit (a) observed at sample site, (b) present along same segment of unconformity, (c) not observed at or near sample site, (d) not present, flow unconformable on >10 m mafic tuff	4 (a)	A251	0.732	1.1616	65.3	40.4±2.0
	5 (a)	A313	1.046	1.7573	58.4	42.7±2.0
	6 (a)	A627	0.947	1.3114	61.7	35.2±1.4
	7 (d)	B658	0.616	0.9204	29.3	38.0±1.6
	8 (c)	B659	1.250	1.9769 1.9630	60.9 60.6	40.1±1.2
	9 (b)	C774	1.220	4.6406 4.6442	49.9 46.9	95±4*
	10 (b)	D191	0.512	0.6812	44.2	33.9±1.3
	11 (c)	D409	1.024	1.5690	40.8	39.0±1.5
	12 (b)	K095	1.158	1.3258 1.4450	64.9 63.0	30.5±1.0*
Sanidine vitrophyre flow overlying several hundred metres of flood basalt with basal red grit	13	A515†	3.086	3.8720 4.0333	59.6 47.3	32.7±1.3
Younger pre-rift sequences						
Gok basalt; (e) basal flow, (f) number of flows above base not known, but less than 5; unconformable on crystalline basement or Permian sandstone	14 (f)	A718	0.963	1.0865	28.2	28.8±1.1
	15 (e)	A735	0.761	1.0386	28.2	34.8±1.3
	16 (f)	B799	1.397	1.6690	59.9	30.5±1.2
	17 (e)	C775	0.954	0.8640	58.7	23.1±1.2*
	18 (f)	D677	0.777	0.9716	22.5	31.9±1.2
Makonnen basalt; (g) basal flow, (h) third flow above base; unconformable on crystalline basement	19 (g)	B683	1.255	1.5549	59.0	31.6±1.2
	20 (g)	B685	0.759	0.9269	42.9	31.1±1.2
	21 (h)	C695	1.068	1.3179	70.3	31.5±1.0
	22 (g)	K002	0.754	0.9699	48.6	32.8±1.2
Fejej basalt, basal flow on crystalline basement	23	C256	0.614	0.7897	55.0	32.8±2.0
Teltele basalt, basal flows; (i) separated from crystalline basement by 6-m tuff layer	24	B125	1.918	1.5726	57.7	21.0±0.8
	25 (i)	B247	0.475	0.3476	38.1	18.7±0.8
	26	B334	1.079	0.8956	28.3	21.2±1.0
	27	B425	0.665	1.4683 1.4019	31.4 32.3	54.7±2.0*
Surma basalt, basal flow on crystalline basement	28	D509	1.210	0.9157 0.9148	30.4 28.4	19.4±0.6
Stefanie Rift; pre-rift hypabyssal phonolite intrusions	29	A033	4.08	2.0639	4.9	13.0±2.4
	30	A034	4.25	2.1130	13.4	12.7±1.0
Post-rift sequence						
Basal basalt flow, outlier on crystalline basement north-east of Lake Turkana	31	A164	0.897	0.1394 0.1504	22.2 18.5	4.2±0.4

$\lambda_\alpha = 4.962 \times 10^{-10}$ yr^{-1}; $\lambda_\varepsilon = 0.58 \times 10^{-10}$ yr^{-1}; ^{40}K/K = 0.01167 atom % (Steiger and Jager[14]).

* Ages considered anomalous.

† Determined on sanidine extract; all other determinations are whole rock.

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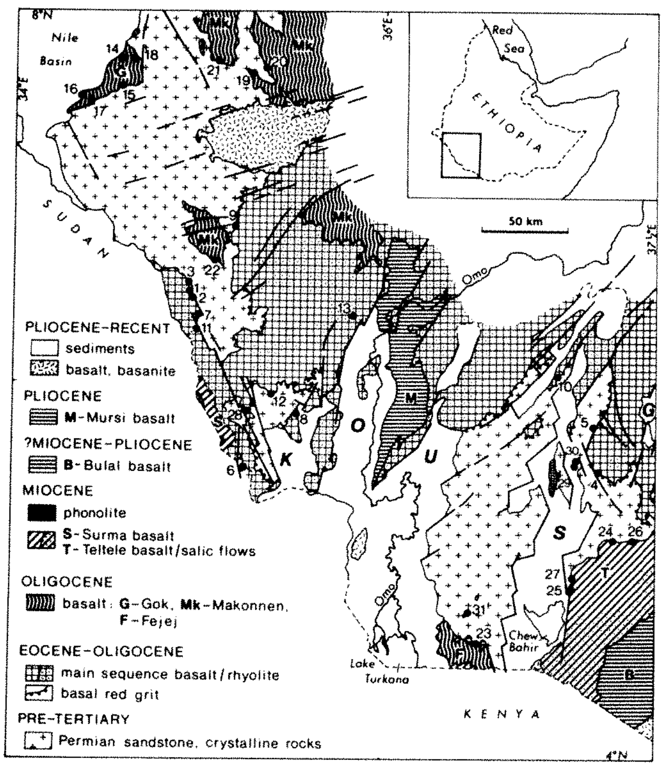

Fig. 1 Geology, southwestern Ethiopia, and location of dated samples. G, Ganjuli graben; S, Stefanie Rift; U, O and K, Usno, Omo and Kibish branches of Turkana Rift.

We conclude: (1) volcanism began as long ago as the middle Eocene and was widespread by the late Eocene in south-western Ethiopia. Early flows were deposited on a peneplain, likely at low elevation. Whether these flows were once continuous with the Ashangi basalts of central Ethiopia, or whether the two relate to separate broad centres of Eocene volcanism is not known. (2) Throughout the Oligocene, a broad arch developed, concave to the north-east, with uplift maxima in the north-west and south-east parts of the area. Oligocene and Miocene lavas covered older flows and overlapped on to basement, in part re-exposed, on top of and flanking the south and west sides of this arch. (3) Major rift structures did not form across this uplift until after Miocene (13–12 Myr) phonolite volcanism, although shallow proto-rifts may have existed somewhat earlier[21]. Rift topography was fully developed by the early Pliocene (5–4.5 Myr). (4) Correlation of Gok and Makkonen basalts, whose base elevations differ by 1.5 km, implies major faulting at the western edge of the Ethiopian Highland. (5) Quaternary volcanism was not restricted to the axial rift zone, but occurrences outside this zone are spatially related to new developing rifts or fracture zones. These discoveries in southwestern Ethiopia require that the pattern of migration of volcanism with time in the Ethiopian region of the Afro-Arabian Rift System be re-assessed.

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Free calcium in *Xenopus* embryos measured with ion-selective microelectrodes

T. J. Rink & R. Y. Tsien

Physiological Laboratory, University of Cambridge, Cambridge, UK

A. E. Warner*

Department of Anatomy and Embryology, University College London, Gower Street, London WC1, UK

A regulatory role for intracellular free calcium has been suggested in cell division[1] and intercellular communication via gap junctions[2,3]. Previous measurements of cytoplasmic free Ca^{2+} during cell division and uncoupling have been made with the Ca^{2+}-sensitive photoprotein aequorin[4,5]. We now report experiments using Ca^{2+}-sensitive microelectrodes to monitor free Ca^{2+} in the cells and intercellular fluid of early embryos of the amphibian *Xenopus laevis*. In addition to measuring basal levels, we have looked for changes in free Ca^{2+} during cell division and during electrical uncoupling of the normally coupled embryonic cells, induced by acidification of the intracellular medium[5–7].

Ca^{2+}-selective microelectrodes, tip size 1–2 μm, were made with a neutral ligand sensor based on that originally described by Oehme *et al.*[8]. The micropipettes were siliconised with tributylchlorosilane vapour at 200 °C. The sensor contained 2.4% tetraphenylphosphonium bis(1,3-diethyl-2-thiobarbiturate)trimethineoxonol in place of the 1% sodium tetraphenylborate commonly used in this type of electrode. Details of the fabrication, calibration and properties of these electrodes will be published elsewhere (R.Y.T. and T.J.R., in preparation). In the presence of 0.1 M KCl, the electrodes showed ideal (Nernstian) responses between *p*Ca 3 and *p*Ca 6 (1 mM to 1 μM free Ca^{2+}), 12–20 mV between *p*Ca 6 and 7, and 4–8 mV between *p*Ca 7 and 8. Interference from H^+ and Mg^{2+} was negligible. At *p*Ca 7 and 6, alterations in *p*H between 7.8 and 6.5 produced no detectable electrode response. Elevation of free Mg^{2+} from 0 to 5 mM or of Na^+ from 0 to 20 mM had less effect on the electrode potential than raising free Ca^{2+} from 0 to 0.1 μM. The free Na^+ in *Xenopus* embryonic cells is probably no more than 20 mM (ref. 9). High concentrations of CO_2 did not alter the electrode responses.

Basal measurements were made on embryos between the 2-cell and 64-cell stages. The mean value for cytoplasmic Ca^{2+} was *p*Ca 6.5 (0.32 μM) ± 0.12 (mean ± s.e.m.) from 12 reliable

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* To whom reprint requests should be addressed.

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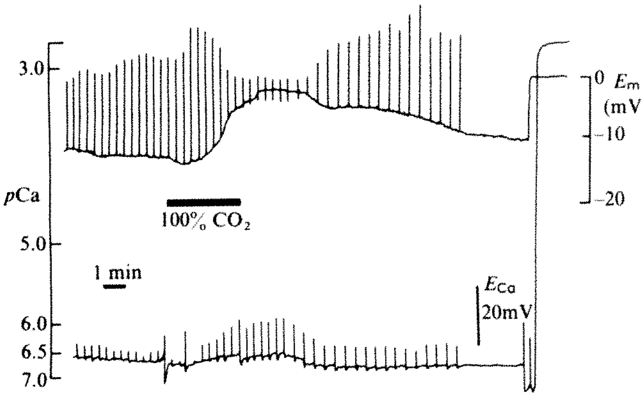

Fig. 1 Membrane potential, electrical coupling and intracellular free Ca^{2+} in a 16-cell *Xenopus* embryo during exposure to 100% CO_2. Three intracellular microelectrodes were used; one voltage recording electrode and one Ca^{2+}-sensitive electrode in one cell, and a third electrode in an adjacent cell which injected repetitive, rectangular current pulses (10 nA, 1 s duration). Upper trace gives the membrane potential, the lower trace the potential E_{Ca}, measured as the difference between the potentials recorded by the voltage and Ca^{2+}-sensitive electrodes. Electrotonic potentials produced by current injected into the adjacent cell are shown as upward deflections on the membrane potential record. The height of these deflections gives a simple indication of the degree of current spread from cell to cell. The changes in input and junctional resistances during CO_2 treatment have been documented[6,7]. The deflections on the lower trace reflect capacitative artefact which is picked up to a greater degree by the Ca^{2+} electrode because of its long electrical time constant. The frequency of the injected current pulses was reduced during the latter part of the trace to improve resolution of baseline E_{Ca}. The right-hand vertical axis shows the membrane potential. The left-hand vertical axis shows the *in vitro* calibration for this Ca^{2+} electrode. All *p*Ca calibrating solutions contained 0.1 M KCl. The required *p*Ca was set by 5 mM Ca and 10 mM ligand, nitrilotriacetic acid for *p*Ca 5.0, *N*-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetic acid (HEEDTA) for *p*Ca 6.0 and 6.5, and EGTA for *p*Ca 7.0. The solutions also contained 10 mM TAPS, HEPES, HEPES and MOPS respectively, and were titrated with KOH to the correct *p*H values (8.42, 7.70, 8.20 and 7.29) to give the desired *p*Ca using stability constants from ref. 18. The embryo was continuously superfused with a Holtfreter solution containing 60 mM NaCl, 1 mM KCl, 1.8 mM $CaCl_2$ and 2 mM ACES, *p*H 6.5. During the period marked by the bar, the superfusion solution was changed to bicarbonate-buffered Holtfreter solution (40 mM $NaHCO_3$ replacing 40 mM NaCl) bubbled with 100% CO_2. After 3 min of exposure to CO_2, the cell had depolarised and the voltage deflections had completely disappeared, only a biphasic capacitative artefact remaining on the trace. The lower, E_{Ca}, trace shows a small increase in intracellular Ca^{2+}. On return to the initial Holtfreter solution electrical coupling and E_{Ca} recovered. The current pulses were stopped, and first the voltage and then the Ca^{2+}-selective electrode were withdrawn. The recording was deliberately terminated before the membrane potential had reached its original level to recalibrate the Ca^{2+} electrode as soon as possible after the baseline *p*Ca level had been re-established.

impalements in 10 embryos from 7 different batches. The lowest Ca^{2+} levels in this series were *p*Ca 7.3 and 7.1. As most of the errors and artefacts in this type of experiment will lead to elevations in free Ca^{2+}, the value in undisturbed cells is likely to be in the region of 0.1 μM. This is in line with values for free Ca^{2+} obtained in a variety of other cells using all available techniques[10-12]. It contrasts with the ~7 mM total Ca^{2+} in these embryos[9]. Free Ca^{2+} in the intercellular fluid, which lies in the blastocoel cavity, came to 1.12 ± 0.04 mM (±s.e.m., $n = 9$).

No changes in free Ca^{2+} were detected during the cell cycle; in some cases the electrodes remained in one cell throughout two complete cleavages. Further evidence for the measured *p*Ca level and lack of transients comes from complementary experiments in which eggs were injected with calcium buffer solutions to try to set and maintain intracellular free Ca^{2+} levels (ref. 4 and below). Cells buffered to less than 10 nM Ca^{2+} did not divide, suggesting that the electrode measurements of ~0.1 μM Ca^{2+} in uninjected, dividing cells is not a gross overestimate. Cells buffered at around 0.1 μM calcium did divide, suggesting that transients above this level are not essential.

Two experimental procedures reported to block the flow of electrical current via gap junctions from one cell to another are elevation of intracellular free Ca^{2+} (refs 2, 3) and intracellular acidification with CO_2-rich external solutions[6,7,13]. It is known that raising intracellular Ca^{2+} can produce a fall in intracellular *p*H (*p*H_i)[14], and conversely in some preparations lowering *p*H_i may increase free Ca^{2+} (refs 5, 15). The question then arises whether Ca^{2+} and H^+ act independently on the gap junctions or whether one ion acts via the other. To try to examine this question directly, free Ca^{2+} levels were measured in 16- to 64-cell stage embryos during exposure to 100% CO_2. This level of CO_2 almost invariably uncouples cells in these embryos[6,7]. In a few experiments electrical coupling was monitored along with free Ca^{2+}; in others the depolarisation known to accompany the uncoupling was used to mark the effect of CO_2 on the cells. Figure 1 shows an experiment on a 16-cell embryo with an initial *p*Ca of 6.7. On exposure to 100% CO_2 (with almost constant external *p*H), the cell depolarised and current flow from cell to cell was abolished. The *p*Ca increased to about 6.45 (free Ca^{2+} elevation from 0.2 μM to 0.35 μM). After removal of the CO_2 these changes reversed. Similar small elevations of *p*Ca were seen in other experiments. In six trials the mean initial *p*Ca was 6.4 ± 0.13 (±s.e.m.) and during exposure to 100% CO_2 the mean peak level of *p*Ca was 6.19 ± 0.1 (0.63 μM). Thus lowering *p*H_i from about 7.8 to 6.5 (ref. 7) modestly raises free Ca^{2+} to less than 1 μM. The measurement of these small elevations in free Ca^{2+} relies on the virtual insensitivity at *p*Ca 6–7 of the electrodes to *p*H changes over the experimental range, a technical advantage not shared by other techniques in use[5,16,17]. The source of the Ca^{2+} transient has yet to be established. Some Ca^{2+} could enter the cells across the plasma membrane from the

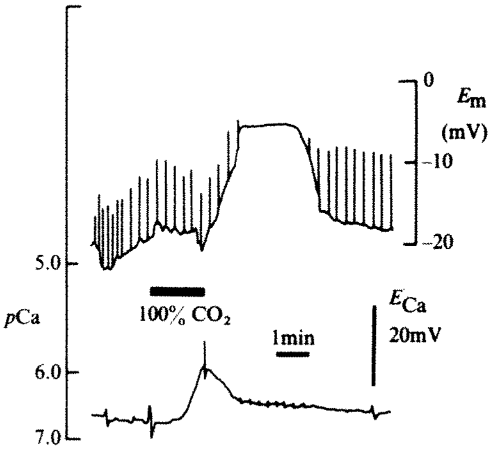

Fig. 2 Effect of 100% CO_2 on electrical coupling, membrane potential and E_{Ca} in one cell of an embryo at the 32-cell stage which was preinjected with CaEGTA. All experimental conditions were as in Fig. 1. A small amount, about 5% of the cell volume, of 200 mM CaEGTA solution containing some fast green FCF stain was injected into one half at the 2-cell stage. The injected half was recognised at later stages of development by the green stain and by the changes in pigment distribution which occurred during CO_2 treatment only in the injected half. Before withdrawing the electrode this embryo was again challenged with 100% CO_2 (not illustrated). This produced a slightly smaller and less prompt rise in *p*Ca, but the cells did not uncouple. The absence of uncoupling may have been because the embryo suffered cumulative damage as a result of simultaneously raising Ca_i^{2+} and H_i^+. Normal embryos (not injected with CaEGTA buffer) uncouple repetitively in response to successive changes with 100% CO_2 (ref. 7).

blastocoel fluid; equally acidification may release Ca^{2+} from intracellular binding sites. It is even possible that CO_2 might release part of the bound Na^+ of these cells[9], which might then contribute to the electrode response. If so, then the true Ca^{2+} transient would be even smaller than the figures quoted above.

Could the CO_2 have induced larger elevations of Ca^{2+} that escaped detection? To check that large transients could be seen, free Ca^{2+} levels were measured in embryos which had been injected with 200 mM CaEGTA (1:1 Ca to EGTA) to a final concentration of 5–10 mM. The injection was made into one cell of a two-cell embryo, which was then left to cleave to the 32- or 64-cell stage. At the normal pH_i of 7.8, almost all the injected Ca^{2+} would stay complexed to the EGTA as the effective dissociation constant was 10 nM (ref. 18). The CaEGTA then would have very little buffering power and could hardly change the free Ca^{2+} inside the cell. On acidification to, say, pH 6.5, the effective dissociation constant would rise to 4 μM, high enough for much of the Ca^{2+} to be unloaded and for the EGTA now to contribute significantly to the total calcium buffering inside the cell. The free Ca^{2+} should go to some value near 4 μM (pCa 5.4) depending on the relative buffer capacities of the cytoplasm and the injected EGTA. Figure 2 shows an experiment of this type. On the application of 100% CO_2 there was a marked, abrupt increase in free Ca^{2+} from pCa 6.6 to 5.9 (0.25 to 1.25 μM). In seven such trials the mean initial and peak pCa values were 6.28 ± 0.16 and 5.76 ± 0.06. This latter value is reasonably close to the pCa 5.4 effective dissociation constant of the EGTA, considering that the intracellular Ca^{2+} buffering is not quantitated and that the change in pH_i is somewhat variable[7]. Clearly the electrodes are able to record large, experimentally induced elevations of free Ca^{2+}. As shown in Fig. 2 the application of CO_2 depolarised and uncoupled the cells; the Ca^{2+} transient was ending as the uncoupling began.

It seems, then, that 100% CO_2 produces elevations of free Ca^{2+} well below the ~50-μM level[2,19] considered to be necessary to abolish current flow from cell to cell. Again one could ask if CO_2 might have caused Ca^{2+} increases localised in every experiment far from the tip of the Ca^{2+} electrode. Two lines of evidence argue against such a possibility. First, Rose and Rick[5] have examined the effect of CO_2 on the luminescence of aequorin microinjected into *Chironomus* gland cells. In the 40% of cells which showed an increased glow, that increase was diffusely distributed. Second, we have injected *Xenopus* embryos with a new Ca^{2+} buffer, 1,2-di(*o*-aminophenoxy)ethane-*N,N,N′-N′*-tetraacetic acid[20], whose affinity for Ca^{2+} (log stability constant = 6.97) is practically independent of pH and Mg^{2+} in the physiological range. The final concentrations of ~10 mM buffer half-saturated with Ca^{2+} should have clamped the intracellular calcium near 0.1 μM. Of 45 such embryos, 64% continued to cleave, although often at a slower than normal rate. In 4 out of 5 injected embryos, the cells uncoupled when challenged with 60% or 100% CO_2. These experiments suggest that *Xenopus* embryos can still be uncoupled when Ca^{2+} transients are greatly damped.

Thus, our results show the levels of free Ca^{2+} in cells of the early amphibian embryo to be in the region of 0.1 μM. They suggest that cell division is not accompanied by transient changes in free Ca^{2+}. A minimum level of Ca^{2+} seems to be necessary for cell division to occur[4], but Ca^{2+} does not have a regulatory role in these cells. The results also support the view that CO_2-induced uncoupling of cells in this preparation is not mediated by alterations in free Ca^{2+} levels. However, the relative importance of Ca^{2+} and H^+ in the control of gap junction permeability may vary from tissue to tissue[21].

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Host gene influences sensitivity to interferon action selectively for influenza virus

O. Haller, H. Arnheiter, J. Lindenmann & I. Gresser

Institute for Medical Microbiology,
Division of Experimental Microbiology,
University of Zürich, POB, CH 8028 Zürich, Switzerland

Institut de Recherches Scientifiques sur le Cancer,
POB, 94800 Villejuif, France

Several examples of genetically determined resistance to different viruses have been studied in mice[1]. Inborn resistance due to the allele *Mx* in A2G mice may be characterised as follows: resistance is specific for members of the orthomyxo family (influenza virus)[2]; it is expressed in different organs and cells[2–6]; and it is independent of a functioning immune system[3,4,7]. The only means of rendering resistant mice fully susceptible to influenza virus has been treatment with potent anti-interferon serum[8], indicating that interferon is important in resistance. Interferon is generally thought to inhibit the multiplication of all animal viruses relatively indiscriminately. Therefore, it had seemed unlikely that interferon was involved in this resistance, as A2G mice were selectively resistant to influenza virus albeit as sensitive as control mice to several other viruses[4]. There are, however, examples in which the genetic control of the production of interferon or the sensitivity to interferon action is expressed selectively for a given virus. Thus, De Maeyer and coworkers have shown that four different *If* loci control the amount of circulating interferon produced after injection of mice with Newcastle disease virus (NDV)[9], mouse mammary tumour virus[10], or Sendai virus (personal communication). Hanson *et al.*[11] found that cultures of cells from C3H RV mice, resistant to Arbo B virus infection, were much more susceptible to the inhibitory effect of interferon when tested with Arbo B viruses than cultures taken from susceptible C3H mice. We present here results, showing that cultures of macrophages from mice resistant to orthomyxoviruses (*Mx* positive) can be protected by much smaller amounts of interferon than are necessary to protect cultures from susceptible mice when these cultures are infected with influenza virus; whereas no difference in sensitivity to interferon is observed when cultures are infected with vesicular stomatitis virus (VSV) or encephalomyocarditis virus (EMC). We believe that these results showing a genetic control of sensitivity to interferon action specific for a given virus are related to the *in vivo* resistance of A2G mice to influenza virus. If applicable to man, these results may also be of importance in understanding individual variations in sensitivity to viral infections.

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Table 1 Viral susceptibility of cultured macrophages

Macrophage		Titre of virus ($\log_{10}$ TCID$_{50}$ per ml)		
Origin	Genotype	M-TUR	EMC	VSV
CBA/J	(+/+)	8.3	7.8	8.5
A/J	(+/+)	7.8	ND	9.0
A2G	(Mx/Mx)	7.8	7.8	8.8
(A2GxA/J)F$_1$	(Mx/+)	8.3	ND	ND

A/J and CBA/J mice were from G. L. Bomholtgård. Inbred A2G mice, homozygous for the resistance allele Mx^2 and heterozygous (A2G × A/J)F$_1$ hybrids were bred locally. Macrophage cultures were prepared as described previously[6,8]. Briefly, peritoneal macrophages from 8- to 12-week-old mice stimulated 3 days previously by intraperitoneal (i.p.) injection of 2 ml of thioglycollate medium were collected. 1.5×10^6 viable cells in 1.0 ml of RPMI 1640 medium were plated into 16-mm wells of 24-well tissue culture cluster plates. After frequent washings to remove non-adherent cells, macrophages were incubated for 4 days in serum-free medium. Thereafter, RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum was added and adherent cells were kept in culture for a total of 21 days. Macrophage cultures were then washed and 0.5 ml of serial 10-fold viral dilutions in serum-free culture medium were added to quadruplicate wells. Cytopathic effects for the following three viruses were assessed 72 h after infection: *M-TUR*, an avian influenza A virus strain derived from A/Turkey/England/63 (Hav$_1$, Nav$_3$)[17] adapted to grow in mouse peritoneal macrophages *in vitro*[6]. Stock allantoic fluid had a haemagglutination titre of 1:640 and $10^{8.7}$ mean tissue culture infective doses (TCID$_{50}$) per ml when assayed on primary chick embryo fibroblast (CEF) cultures. *VSV*, serotype Indiana was grown and stored as previously described[15]. VSV stock allantoic fluid had a titre of $10^{8.7}$ TCID$_{50}$ per ml on L$_{929}$ cells. *EMC* virus[18] was propagated in monolayer cultures of L$_{929}$ cells and was adapted to grow in mouse macrophages by five consecutive passages in the murine macrophage monocytic tumour cell line J-774 (ref. 19) followed by two passages in peritoneal macrophages from CBA/J mice cultured for 3 weeks. Virus stocks consisted of cell free medium from 24-h cultures of J-774 cells and had a titre of $10^{8.5}$ TCID$_{50}$ per ml for L$_{929}$ cells. ND, not determined.

Macrophages collected from the peritoneal cavity of A2G mice are resistant to influenza virus when first placed in culture[6], but become susceptible with time[8]. We therefore cultivated peritoneal macrophages for 3–4 weeks, at which time they were highly susceptible to the three test viruses used, macrophage-adapted orthomyxovirus strain (M-TUR), a rhabdovirus (VSV) or a picornavirus (EMC) (Table 1). At 3 to 4 weeks, cultures of macrophages were incubated with graded doses of mouse interferon for 18 h before viral challenge. Two preparations of mouse interferon induced by NDV in mouse C-243 cells were used: (1) partially purified to 1×10^7 reference units per mg protein[12]; (2) highly purified by sequential chromatography on sheep anti-interferon antibody[13] and CM-sepharose[14] columns to 5×10^8 reference units per mg protein (a gift from Dr M. Aguet).

Figure 1 illustrates the effects of 40 and 400 reference units of partially purified interferon on the multiplication of influenza virus M-TUR in macrophages from CBA/J (susceptible) and A2G (resistant) mice. At high (Fig. 1 *a*, *b*) and low (Fig. 1 *c*, *d*) multiplicities of infection, both interferon doses markedly inhibited virus replication in A2G mouse macrophage cultures (Fig. 1 *b*, *d*), whereas even 400 interferon units exerted only a slight inhibition or delay of viral multiplication in CBA/J macrophage cultures (Fig. 1*a*, *c*).

Figure 2 shows the effect of different amounts of highly purified interferon on the multiplication of M-TUR virus and EMC virus in cultures of macrophages from (A2G × A/J)F$_1$ mice (heterozygous for the gene *Mx* and thus genetically resistant to influenza virus but susceptible to EMC virus[2]) and from A/J mice (susceptible to both viruses). The inhibitory effect of interferon on the multiplication of EMC virus was similar in cultures of macrophages from these two strains of mice. In contrast, a marked difference was observed in the degree of inhibition by interferon of the multiplication of M-TUR virus in

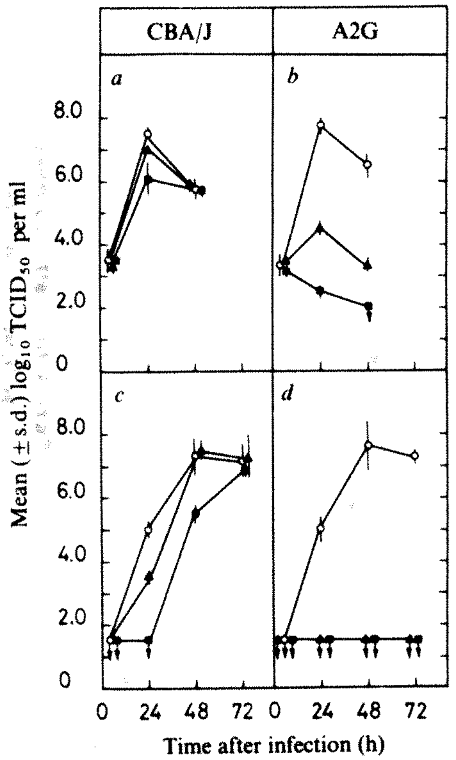

Fig. 1 Inhibition by interferon of multiplication of M-TUR virus in cultures of macrophages from susceptible CBA/J mice (*a*, *c*) or resistant A2G mice (*b*, *d*). Peritoneal macrophages cultivated for 3 weeks in culture were incubated for 18 h with 40 (▲) or 400 (■) reference units per ml of partially purified mouse interferon. Interferon pretreated and untreated cells (○) were then washed and infected with M-TUR virus at multiplicities of either 5 (*a*, *b*) or 0.005 (*c*, *d*). After incubation for 60 min at 37 °C the virus inoculum was removed by repeated washings. Cell-free medium from individual cultures was assayed for infectivity on CEF cultures at the times indicated. Each point indicates the mean TCID$_{50}$ from three cultures with standard deviation.

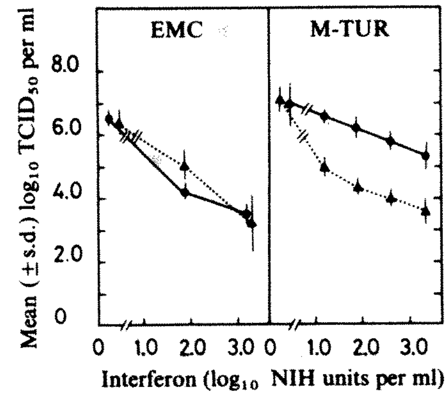

Fig. 2 Effects of highly purified interferon on multiplication of EMC and M-TUR virus in macrophages from resistant or susceptible mice. Peritoneal macrophages from resistant (A2G × A/J)F$_1$ mice (Mx/+), (▲ ··· ▲), and from susceptible A/J mice, (+/+), (●——●), were cultured for 3 weeks and incubated for 18 h with various doses of highly purified interferon. Thereafter, cultures were washed and infected at a multiplicity of 5 with either M-TUR or EMC virus. After an incubation period of 60 min at 37 °C the virus inoculum was removed by repeated washings. The viral titre of cell-free medium from four individual cultures per interferon dilution was determined 18 h after viral infection (at the time of peak virus production in control cultures). EMC virus was titred in monolayer cultures of L$_{929}$ cells and M-TUR virus in CEF. Mean TCID$_{50}$ values and standard deviations are indicated. The differences in yields of M-TUR virus between interferon-treated +/+ and Mx/+ macrophage cultures were statistically significant ($P < 0.005$ for all interferon doses used).

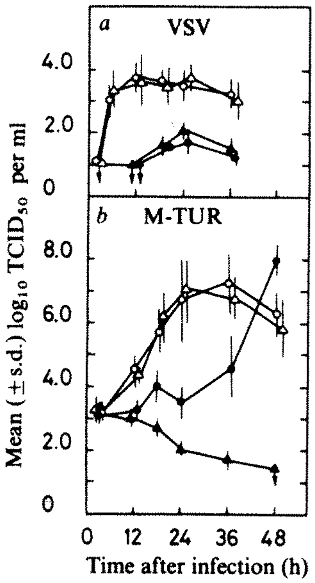

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Fig. 3 Effect of interferon on the multiplication of M-TUR and VSV in macrophage cultures from backcross mice segregating for the allele *Mx*. (A2G×C57BL/6J)F$_1$ were backcrossed with C57BL/6J mice. The offspring were characterised as carriers (Mx/+) or noncarriers (+/+) of the allele *Mx* as described[6]. After 3 weeks in culture, peritoneal macrophages obtained from individual Mx/+ or +/+ backcross mice were incubated for 18 h with 20 (*a*) or 40 (*b*) reference units of partially purified mouse interferon. Cells were then washed and infected with either VSV (*a*) or M-TUR (*b*) at a multiplicity of 5. After incubation for 90 min at 37° C the virus inoculum was removed by repeated washings. Cell-free medium from individual cultures was titrated on CEF monolayers at the times after infection indicated. Titres represent mean values from three macrophage cultures of either resistant (Mx/+) or susceptible (+/+) genotype. ○, (+/+) Untreated; ●, (+/+) interferon-treated; △, (Mx/+) untreated; ▲, (Mx/+) interferon-treated.

macrophage cultures from these mice. Thus in A/J cells, 2,000 reference units of interferon resulted in only a 50-fold reduction in the yield of M-TUR virus, whereas the same amount of interferon reduced M-TUR virus yield by 4,000-fold in (A2G×A/J)F$_1$ cells.

In backcrosses segregating for the two alleles at the *Mx* locus, the susceptibility (+/+) or resistance (Mx/+) to orthomyxoviruses of individual mice can be determined by typing peritoneal macrophages[6]. Interferon had a similar and profound inhibitory effect on the multiplication of VSV in both Mx/+ and +/+ cells (Fig. 3*a*) (Mx/+ and +/+ mice are equally susceptible to VSV)[15]. In contrast, interferon slightly delayed multiplication of M-TUR virus in +/+ cells, but markedly inhibited its multiplication in Mx/+ cells (Fig. 3*b*).

We have not yet determined which step in influenza virus replication is affected in interferon treated *Mx*-bearing cells. Our preliminary results indicate that neither virus adsorption nor virus penetration are involved but that viral protein synthesis seems to be blocked at an early stage (M. Horisberger *et al.*, in preparation).

Most laboratory strains of mice do not carry the *Mx* gene, and thus support influenza virus multiplication despite exposure to relatively large amounts of interferon. However, there are indications that resistance of wild mouse populations to orthomyxoviruses is widespread (O.H., unpublished). We do not know whether this resistance is also interferon mediated (as in our A2G mice) but it is of interest that interferon also seems to be involved in mouse resistance to flaviviruses[11], and that wild mice are also frequently found to be resistant to these viruses[16]. The phenomenon of genetic resistance to viral infections mediated by interferon may therefore be more general than has heretofore been supposed. It is an interesting example of cooperation between a nonspecific extrinsic factor (interferon) with an intrinsic element (the resistance gene) resulting in a degree of antiviral specificity. Thus, the resistance gene can be envisaged either as modulating the antiviral state induced by interferon or as coding for an antiviral mechanism activated by interferon. Alternatively, it might be speculated that the seemingly nonspecific antiviral state generally observed in interferon-treated cells would be composed of a multitude of specific antiviral mechanisms each of which might be coded for by a unique resistance gene of the host cell. Allelism at such resistance loci would then account for various degrees of protection induced by interferon.

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Spontaneous stomach ulcer in genetically mast-cell depleted *W*/*W*^{v} mice

M. Shimada, Y. Kitamura*, M. Yokoyama, Y. Miyano, K. Maeyama, A. Yamatodani & Y. Takahashi

Institute for Cancer Research, Department of Pharmacology and
Institute of Higher Nervous Activity, Medical School,
Osaka University, Kita-ku, Osaka, 530 Japan

M. Tatsuta

Department of Gastroenterology, Center for Adult Diseases,
Higashinari-ku, Osaka, 537 Japan

Histamine has been implicated in the pathogenesis of gastroduodenal ulcers[1,2]. Since mast cells contain a considerable amount of histamine as well as heparin and other biologically active substances[3], it seems reasonable to speculate that they are involved in this process[4,5]. In fact, development of gastroduodenal ulcers was reported in dogs with mastocytoma[6], and an increase in mast-cell number was described in the vicinity of human gastroduodenal ulcers[7,8]. Y.K. *et al.*[9] have recently found that the number of mast cells in a unit length of the skin of *W*/*W*^{v} mutant mice is less than 1% of the value for the congeneic +/+ mice and that no mast cells are detected in the intestinal canal of *W*/*W*^{v} mice. Therefore, this mutant mouse was used to investigate the role of mast cells in the induction of gastroduodenal ulcers. As a preliminary experiment, we examined the stomach and duodenum of *W*/*W*^{v} mice without any treatments. Unexpectedly, we found the spontaneous development of perforating stomach ulcers in *W*/*W*^{v} mice, suggesting that mast cells do not necessarily have aggravating effects on the production of stomach ulcers.

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*To whom correspondence should be addressed at: Institute for Cancer Research, Medical School, Osaka University, Nakanoshima 4-chome, Kita-ku, Osaka, 530 Japan.

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Table 1 Incidence of stomach ulcers in WBB6F$_1$ mice of different genotype, sex and age

		Incidence of ulcers in each age (no. (%))	
		1–2 months	2–6 months
W/W^v	Male	9/18 (50)	11/24 (46)
W/W^v	Female	21/28 (75)	4/16 (25)
$W/+$	Male and female	0/19 (0)	0/22 (0)
$W^v/+$	Male and female	0/18 (0)	0/20 (0)
$+/+$	Male and female	0/23 (0)	0/21 (0)

Mice of WBB6F$_1$(WB $-$ $W/+$ $\times$ C57BL/6 $-$ $W^v/+$)$-$(W/W^v, $W/+$, $W^v/+$, $+/+$) were used. WBB6F$_1$ mice were raised in our laboratory using parental stocks originally obtained from the Jackson Laboratory, Bar Harbor. The *W* genotype of all animals was inferred from the pattern of white spots and dilution of coat colour[10]. In addition to mast-cell depletion, the mice of W/W^v genotype show severe macrocytic anaemia, sterility and depletion of hair pigment[10]. Mice were kept in conventional animal facilities with *ad libitum* access to Oriental Lab Chow (Oriental Yeast Co.) and tapwater.

At an age of 1 to 6 months, W/W^v mutant mice and their littermates were killed; the abdominal wall was opened; deformity of pyloric region and adhesion between pylorus and adjacent organs were inspected. The stomach and duodenum were removed as a whole; the stomach was split along the greater curvature. After macroscopic examination, the stomach and duodenum were fixed in 10% buffered formalin (*p*H 7.2). Ordinary paraffin sections (5 μm thick) were made, and stained with haematoxylin and eosin to confirm diagnosis, or with toluidine blue to determine the number of mast cells, or with the periodic acid Schiff reaction to count the number of mucous cells. In most cases, subserial sections were made to determine the size of the ulcer when found. The diameter of the ulcer was measured in the largest cross-section of the ulcer using an occular micrometer attached to an eyepiece of the microscope.

Gastric secretions were collected for 4 h by the method described by Shay *et al.*[11]. The volume of secretion was measured, the acid concentration was titrated with 0.1 M NaOH, and the acid output was calculated. Pepsin concentration was determined according to the method reported by Anson and Mirsky[12]. Histamine was separated by column chromatography after dansylation from various parts of the stomach and its concentration was determined with the fluorimeter[13]. Distribution of enterochromaffin-like cells was examined with histofluorescence technique[14] with or without prior injection of L-dopa (100 mg per kg body weight) (ref. 15).

In about a half of the untreated W/W^v mice, chronic ulceration was found in the prepyloric region of the stomach. The incidence of ulcers was not affected by age in males, but was higher in young females (1–2 months) than in older ones (2–6 months) (Table 1). The size range of the ulcers was 0.6–4.5 mm and the median size was 2.2 mm. In some cases, the lesion was so wide that it occupied the whole prepyloric region, but it did not extend into the fundic part even in such cases. The ulcer frequently perforated, and adhesion of the pyloric region to the liver and pancreas was observed. Histologically, the ulcer crater contained necrotic debris and was sharply demarcated and infiltrated by inflamatory cells. The muscular layers were interrupted by granulation tissue and fibrous connective tissue (Fig. 1). When the section was stained with toluidine blue, no mast cells were detected either in the vicinity of the ulcer or in other parts of the stomach and duodenum. In contrast with the high incidence of ulceration in mast-cell depleted W/W^v mice, no ulcerative lesions were detected in the stomach and duodenum of $W/+$, $W^v/+$, and $+/+$ littermates which have a considerable number of mast cells in the stomach (Table 1).

Table 2 Number of various types of cells in the gastric mucosa of WBB6F$_1$-W/W^v and the congeneic $+/+$ mice

Cell type	No. of cells per gland (mean ± s.e.) W/W^v with ulcer	W/W^v without ulcer	$+/+$
Parietal cell	20 ± 3 (10)	18 ± 1 (15)	17 ± 2 (12)
Chief cell	15 ± 4 (10)	15 ± 2 (15)	12 ± 1 (12)
Surface mucous cell	23 ± 2 (10)	21 ± 1 (15)	21 ± 1 (12)
Neck mucous cell	7 ± 1 (10)	6 ± 1 (15)	7 ± 1 (12)
Pyloric mucous cell		33 ± 2 (7)	33 ± 1 (10)

Number of mice is shown in parentheses.

Table 3 Mast-cell number and histamine concentration in various parts of the stomach of WBB6F$_1$-W/W^v and the congeneic $+/+$ mice

Part of stomach	No. of mast cells*† (per cm of section) W/W^v	$+/+$	Histamine concentration* (nmol per g of wet tissue) W/W^v	$+/+$
Forestomach	0 (9)	172 ± 16 (11)	0.6 ± 0.4 (4)	78 ± 16 (4)
Fundic region	0 (9)	139 ± 11 (11)	57 ± 7 (5)	123 ± 12 (3)
above muscularis mucosae	0 (9)	205 ± 1 (11)		
beneath muscularis mucosae	0 (9)	134 ± 11 (11)		
Prepyloric region	0 (9)	62 ± 8 (11)	15 ± 4 (4)	
above muscularis mucosae	0 (9)	25 ± 2 (11)		
beneath muscularis mucosae	0 (9)	37 ± 7 (11)		21 ± 2 (4)

* Mean ± s.e., number of mice is shown in parentheses.

† See ref. 9 for the method used to determine the number of mast cells.

Since peptic ulcers are thought to occur as a consequence of an imbalance between the factors stimulating acid secretion and the factors protecting the gastric mucosa from acid/peptic digestion[16], the numbers of parietal cells, chief cells and mucous cells were counted in the stomachs of the $+/+$ mice and those of W/W^v mice with or without stomach ulcers. As shown in Table 2, the numbers of parietal cells, chief cells and mucous cells in the $+/+$ mice did not differ significantly from the values observed in W/W^v mice with or without the ulcers. Moreover, when the gastric juice was examined, there was no difference in the rates of acid and pepsinogen secretion between the $+/+$ and W/W^v mice.

As the normal acidity was observed in the stomach of mast-cell depleted W/W^v mice and as histamine is known to be a stimulator of acid secretion[16], the concentration of histamine was evaluated in the stomach of W/W^v and congeneic $+/+$ mice. The number of mast cells was counted in various parts of the stomach to compare with the concentration of histamine. Since the concentration of histamine in the forestomach of the

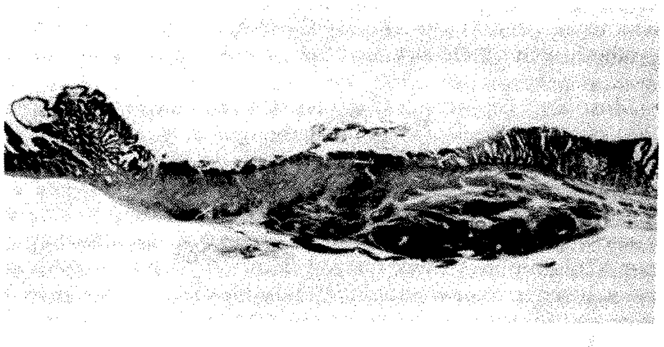

Fig. 1 Histological section of a chronic ulcer localised in the prepyloric region of the stomach of the untreated WBB6F$_1$-W/W^v mice (stained with haematoxylin and eosin).

W/W^v mice was less than 1% of the value of the +/+ mice, the histamine content seemed to be proportional to the number of mast cells in the forestomach (Table 3). In contrast, a considerable amount of histamine was detected in the glandular stomach of mast-cell depleted W/W^v mice (Table 3), suggesting the presence of histamine in certain types of epithelial cells located in the glandular stomach of the W/W^v mice. Examination with the fluorescence microscope revealed that the distribution and number of enterochromaffin-like cells in the fundic mucosa of the W/W^v mice were not significantly different from those observed in the congeneic +/+ mice. The prior injection of L-dopa increased the number of fluorescent cells in both W/W^v and +/+ mice.

The present results showed that the stomach ulcer developed spontaneously in mast-cell depleted W/W^v mice and that the glandular stomach of W/W^v mice contained a considerable amount of histamine in spite of its lack of mast cells. Since the number of enterochromaffin-like cells in the fundic mucosa of the W/W^v mice was not different from the value observed in the +/+ mice, histamine detected in the glandular stomach of the W/W^v mice appears to originate from certain types of enterochromaffin-like cells as demonstrated by Håkanson *et al.*[17]. Therefore, there are possibilities that histamine derived from enterochromaffin-like cells may have an essential role in the pathogenesis of gastroduodenal ulcers as postulated by Guth and Code[1] and Lees *et al.*[18] and that mast cells themselves might have inhibiting rather than aggravating effects on the production of ulcers.

The histological features of the ulcers found in the W/W^v mice are very similar to the chronic human ulcers[16]. Although production of acute ulcers in the fundic mucosa of rats by stress[19] and spontaneous development of duodenal ulcers in NZB mice[20] were previously reported, development of chronic ulcers in the prepyloric region is rare in untreated rodents. Therefore, the ulcer in the W/W^v mice may be a useful model of the human disease.

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Two distinct factors are required for induction of T-cell growth

Eva-Lotta Larsson*, Norman N. Iscove† & Antonio Coutinho*

* Department of Immunology, University of Umeå, Umeå, Sweden
† Basel Institute for Immunology, Basel, Switzerland

The molecular and cellular basis of T-lymphocyte activation remains a central question in immunology. The growth of already proliferating T cells is known to depend on T-cell growth factor (TCGF), a physiological mitogen[1]. Noncycling T lymphocytes, however, are not sensitive to TCGF[2]. They require a short contact with mitogenic lectins, such as concanavalin A (Con A)[3] or leukoagglutinin[4] to bind and respond to TCGF, and will thereafter maintain exponential growth for long periods provided that TCGF is not limiting[5]. While the induction of TCGF reactivity results from the direct contact of Con A with resting T cells[3], the lectin-dependent production of TCGF is known to involve two cell types, both present in mouse spleen[1,3,6,7]. One consists of I-A-negative cells, most of which are Thy-1-positive T lymphocytes[1,3], and the other consists of I-A-positive, immunoglobulin-negative, Thy-1-negative cells, most of which are macrophages[1,3]. The nature of the respective contributions of the two cell types, and in particular the cellular origin of TCGF, has not yet been established. We have now established the I-A-negative population as the source of TCGF and show here that macrophages are required to supply a 20,000-molecular weight factor, chemically and functionally distinct from TCGF, which supports the production of TCGF by the I-A-negative cells.

TCGF was recovered in conditioned medium from Con A stimulated mouse spleen cell cultures[5], and assayed by its capacity to maintain proliferation of T-cell blasts[1,8]. These were derived from spleen cell cultures initially stimulated by Con A and subsequently passaged in CM for periods of 3 weeks or more. Where indicated, a cloned TCGF-dependent cell line of specific cytotoxic T lymphocytes was also used[9]. When TCGF from Con A conditioned medium was partially purified by isoelectric focusing and then fractionated on Sephadex, TCGF activity was detected in fractions corresponding to an apparent molecular weight (MW) near 35,000 (Fig. 1). Identical activity profiles were obtained for fractions tested on the two types of T-cell blasts and on resting I-A-negative T cells after a 4-h sensitising pulse of Con A. The active fractions were pooled and used as a source of partially purified TCGF in subsequent experiments.

The proliferative responses of T cells to Con A were then examined in spleen cell populations depleted of I-A-positive cells. As previously found[1,3,7], Con A alone failed to induce proliferation (Table 1). We therefore tested medium conditioned by macrophages for its capacity to replace the I-A-positive cell requirement. The results shown in Table 1 were obtained with a partially purified preparation of medium conditioned by WEHI-3 cells. These are continuously growing cells of BALB/c origin with functional markers characteristic of macrophages[10]. This material supported a strong proliferative response in the presence of Con A, but it was inactive alone. In these culture conditions, partially purified TCGF seemed to exert a similar action, supporting a response in the presence, but not the absence, of Con A. The combination of TCGF and WEHI-3 activity, however, was inactive in the absence of Con A, demonstrating that the WEHI-3 activity cannot replace Con A in its role of sensitising T cells to TCGF.

Although the WEHI-3-derived activity and TCGF behaved similarly in cultures of resting, I-A-negative cells, their distinct functional properties were apparent in cultures of already proliferating, TCGF-dependent T-cell blasts (Table 1). In such cultures, only TCGF was able to sustain proliferation. WEHI-3 activity had no inhibitory effect in combination with TCGF.

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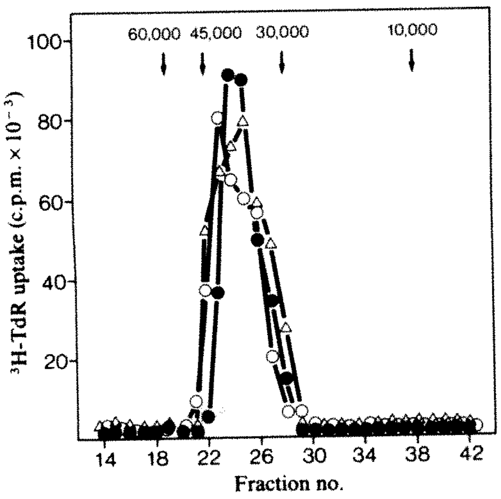

Fig. 1 Growth requirement of T-cell blasts and Con A pulsed purified T cells. Conditioned medium (CM) was obtained by incubating unseparated mouse spleen cells (5×10^6 per ml) for 24 h in the presence of concanavalin A (Con A) (5 μg ml^{-1}) (Pharmacia) in RPMI-1640 medium containing neomycin (50 μg ml^{-1}), HEPES (10 mM), 2-mercaptoethanol (5×10^{-5} M) and 1% fetal calf serum (GIBCO, batch U18710.11). CM (500 ml) was concentrated by dialysis against polyethylene glycol (MW 20,000, Serva), dialysed against buffered saline solution (BSS) and filter-sterilised (0.45 μM). Preparative flat-bed isoelectric-focusing (LKB) in a *p*H gradient from 3.0 to 10.5 was carried out over 24 h at constant power in Ultradex gel (LKB)[20]. Fractions were collected, eluted from small columns, dialysed against BSS, and tested for TCGF activity on T-cell blasts. The TCGF-containing fractions (*p*H 6.0–7.0) were pooled, lyophilised, dissolved in the running buffer, applied to a calibrated Sephadex G-75 superfine column (1.6 × 90 cm), and eluted with 4 mM HEPES containing 0.16 M NaCl and 10 μg ml^{-1} of polyethylene glycol (MW 6,000), *p*H 7.3. Fractions were tested for TCGF activity on Con A-derived T-cell blasts (5×10^4 per ml) (●), a cloned anti-HY (H-2^b) killer cell line[14] (5×10^4 per ml) (○) and I-A-negative resting T cells (2×10^5 per ml) (see legend to Table 1) which had been pulsed with Con A for 4 h (△). The proliferative responses were measured on day 4 and each point represents the mean value of ^{3}H-TdR uptake into DNA in triplicate cultures.

The chemical distinction between TCGF and the WEHI-3-derived activity became apparent when the latter was chromatographed on Sephadex (Fig. 2). Confirming the results in Table 1, no TCGF-like activity was observed in cultures of T-cell blasts with or without Con A (upper panel). However, activity was apparent when tested in cultures of I-A-negative populations containing resting T lymphocytes. There, fractions corresponding to an apparent MW near 20,000, inactive by themselves, supported Con A-dependent proliferation. Thus, TCGF maintains proliferation of T-cell blasts and has an apparent MW near 35,000 on Sephadex. In contrast, the activity from WEHI-3 cells has an apparent MW near 20,000, and is active in cultures of resting T cells but not on Con A-derived T-cell blasts.

These results suggested the possibility that the WEHI-3 product might support the T-cell response to Con A by allowing production or release of TCGF in the I-A-negative cell cultures. TCGF, in turn, would initiate and maintain T-cell growth. To determine whether this was the case, I-A-negative spleen cells were cultured with Con A and the partially purified WEHI-3 activity. After 24 h, the supernatant medium was collected and tested for TCGF activity in cultures of T-cell blasts. The results (Fig. 3) confirm that in the presence of both Con A and the WEHI-3 product, but not with either alone, I-A-negative cells are able to release abundant TCGF activity into the medium. Note that TCGF-dependent T-cell blasts, in contrast to unselected, resting splenic T lymphocytes, are not capable of producing TCGF (or maintaining proliferation) even in the presence of both Con A and the WEHI-3 product. This observation confirms previous negative findings when TCGF-dependent T-cell blasts were incubated with splenic macrophages and Con A (ref. 1).

These experiments show that a soluble macrophage product with an apparent MW near 20,000 is required for Con A-induced elaboration of TCGF. This product is probably identical with that known as lymphocyte activating factor (LAF). This principle is defined as a macrophage-derived factor with an apparent MW near 15,000 which is detected by its capacity to support a proliferative response of thymocytes to low, non-mitogenic concentrations of T-cell lectins such as Con A (ref. 11). LAF is known to be secreted by several macrophage cell lines including WEHI-3 (ref. 12). In this context, the data in Table 1 can be regarded as confirming earlier indications that LAF can substitute for the macrophage requirement in mitogen-dependent T-cell proliferation[13]. Also, our observations confirm and extend those of Smith and coworkers, showing that crude conditioned medium from a macrophage cell line enhanced TCGF production by non-adherent spleen cells in response to Con A (ref. 14). In agreement, we regard the requirement for adherent, I-A-positive cells in T-cell proliferative responses to be now resolved into a requirement for molecules, probably already known as LAF, which are needed only for events leading to TCGF production. Although LAF release can be induced by several macrophage-stimulating agents[15], the nature of the Con A-dependent interaction of T cells and macrophages which leads to production of LAF requires further investigation.

In addition, our results show that TCGF derives from I-A-negative cells rather than macrophages. These observations, together with those of Wagner and Rollinghoff[16] indicating involvement of Lyt-1-positive (helper) T lymphocytes in the generation of TCGF, lead us to favour the view that TCGF originates from these cells. However, our methods do not exclude the possibility that TCGF actually originates from the 3% minority contaminant of I-A- and Thy-1-negative cells, with

Table 1 Growth induction in cultures of purified resting T cells and of T-cell blasts

Addition to test culture	Responses (c.p.m. per culture × 10^{-3} ± s.d.) of anti-I-A-treated and Ig-anti-Ig column passed spleen cells*	Responses (c.p.m. per culture × 10^{-3} ± s.d.) of T-cell blasts†
None	1.3 ± 0.3	0.9 ± 0.4
Con A	2.1 ± 0.9	0.8 ± 0.3
Con A + WEHI-3 activity	31.7 ± 3.6	1.5 + 0.7
WEHI-3 activity	0.6 ± 0.3	1.2 ± 0.4
Con A + TCGF	19.7 ± 3.6	16.1 ± 2.9
TCGF	2.1 ± 0.5	17.3 ± 2.0
TCGF + WEHI-3 activity	1.7 ± 0.4	19.7 ± 3.3

* Spleen cell suspensions from C3H/HeJ mice were treated with monoclonal anti-I-A^k antibodies[18] for 30 min in the cold and passed over a column of plastic beads coated sequentially with mouse Ig and rabbit anti-mouse Ig (ref. 19). The effluent cells, now depleted of I-A-positive and Ig-positive cells, were cultured at 2×10^5 per ml in microtitre plates (0.2 ml per culture), either alone or in the presence of the following reagents as indicated: Con A (5 μg ml^{-1}), partially purified activity from WEHI-3 conditioned medium (see Fig. 2 legend) and partially purified TCGF (see Fig. 1 legend). Mean values of triplicate cultures ± s.d. of incorporated ^{3}H-TdR on day 4 of culture are presented.

† T-cell blasts (5×10^4 per ml), which had been maintained in standard CM for more than 3 weeks, were tested in the same conditions as above. The results from day 3 of culture are presented.

T lymphocytes having some other essential role. Thus, the nature of the lectin and LAF interactions with these cells leading to the release of TCGF remain unclarified. Finally, as TCGF-dependent T-cell blasts, which include killer T cells[5,9,17], are not competent to product TCGF, it seems that different T-cell subpopulations are involved in production and response to TCGF. Although the requirement for TCGF by killer T lymphocytes is clear, a fuller knowledge of the requirements of the T helper cell population is needed.

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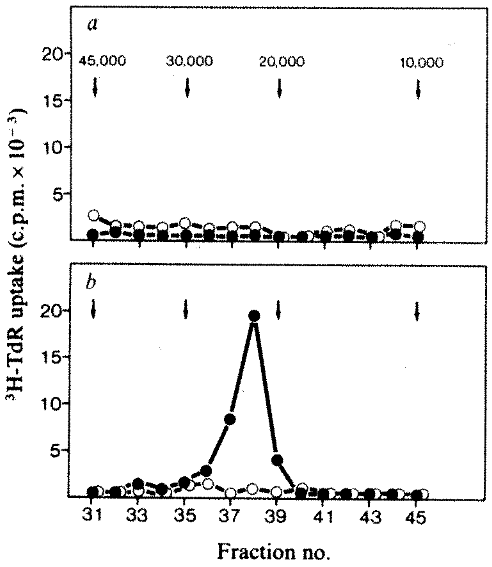

Fig. 2 Fractionation of the growth supporting activity in WEHI-3 CM. WEHI-3 CM was obtained from 5–7-d cultures of WEHI-3 cells in Iscove's modified Dulbecco's medium (IMDM) containing bovine serum albumin (BSA) (750 μg ml^{-1}), human transferrin (30 μg ml^{-1}) and soybean lipid (50 μg ml^{-1}) as described previously[14]. The WEHI-3 CM (760 ml) was concentrated by dialysis against polyethylene glycol (MW 20,000, Serva), applied to a Con A-Sepharose column (2.5×40 cm) (Pharmacia) and eluted with 50 mM Tris HCl *p*H 7.4 containing 0.5 M NaCl and 10^{-4} M Ca^{++}, Mg^{++} and Mn^{++}. The non-interactive material was concentrated over an Amicon Diaflo UM-10 membrane and thereafter applied to a Sephadex G-150 column (2.5×95 cm). Running buffer was as given in Fig. 1 legend. The growth-supporting activity was recovered in the post-albumin fractions, which were pooled and used in the experiments presented in Table 1. This pool was thereafter concentrated over a UM-10 membrane and applied to a Sephadex G-75 superfine column (1.6×90 cm) in the same running buffer as above. The fractions eluted from this column were tested for their capacity to support growth of *a*, T-cell blasts in the presence (●) or absence (○) of Con A (5 μg ml^{-1}); and *b*, purified resting T cells (see legend to Table 1) in the presence (●) or absence (○) of Con A (5 μg ml^{-1}). The proliferative responses were measured and each point represents the mean value of DNA-incorporated ^{3}H-TdR from triplicate cultures.

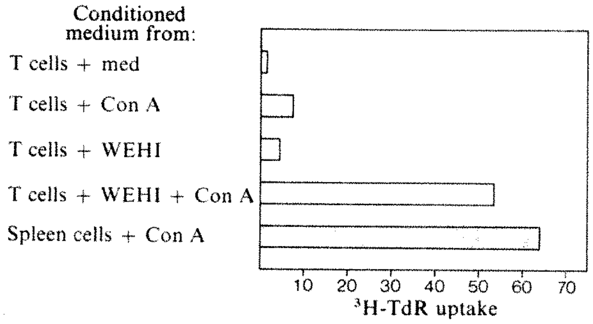

Fig. 3 WEHI-3 activity supports the Con A dependent production of TCGF by purified T cells. Spleen cells depleted of I-A-positive cells were cultured at 2.5×10^6 per ml either alone or with Con A (5 μg ml^{-1}) or partially purified WEHI-3-derived activity (pooled active fractions indicated in Fig. 2). The supernatant medium was tested for TCGF activity in T-cell blast cultures. As a positive control, normal spleen cells were cultured at 5×10^6 per ml in the presence of Con A (5 μg ml^{-1}) for 24 h. The proliferative responses were measured on day 3 of culture and the results are presented as the mean values of ^{3}H-TdR (c.p.m.×10^{-3}) incorporated into DNA in triplicate cultures.

Immunisation against heterologous type II collagen induces arthritis in mice

J. S. Courtenay, Margaret J. Dallman, A. D. Dayan, Angela Martin & Betty Mosedale

Departments of Protein Chemistry and Pathology, Wellcome Research Laboratories, Beckenham, Kent, UK

The induction of polyarthritis in rats by intradermal immunisation with homologous or heterologous type II collagen in complete or incomplete Freund's adjuvant was reported recently by Trentham *et al.*[1]. We have now produced a similar disease in certain strains of mice.

Mice were injected with either type I or type II bovine collagen, prepared using the method of Trentham *et al.*[2], emulsified in complete Freund's adjuvant (CFA) or incomplete Freund's adjuvant (ICFA), in experimental conditions similar to those known to be effective in the rat. The time of onset, and incidence of arthritis were determined by daily clinical examination, regular measurement of the thickness of hind foot pads with a micrometer gauge, and after necropsy, by histological examination of proximal and distal joints in fore and hind limbs, the tail, ear and spinal column.

The findings reported here mainly concern the induction of arthritis, clinically affecting several joints, in the DBA/1 strain of inbred mice (Laboratory Animals Centre, Carshalton). Mice aged 8–15 weeks were initially injected intradermally in the back with bovine type I or type II collagen, or the acetic acid solvent for the collagens, emulsified in CFA or ICFA. Mice were challenged 3 weeks later with an intraperitoneal injection of the same type of collagen, or of acetic acid alone.

The first sign of swelling and redness of a fore or hind paw was detected after 23 d in mice injected with type II bovine collagen in CFA. The swelling progressed to a maximum at different times in individual joints and then gradually regressed, although the joints still appeared badly damaged on histological

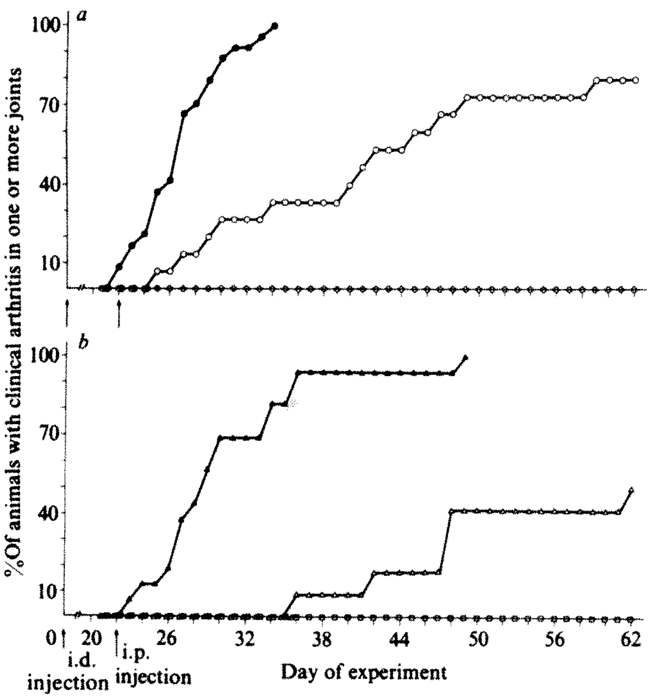

Fig. 1 Incidence and time of onset of arthritis in DBA/1 mice. DBA/1 mice were injected intradermally into 4–6 sites on the back with 100 μg of bovine type II collagen, dissolved in 0.1 ml of 0.1M acetic acid and emulsified in an equal volume of CFA (0.5 mg ml^{-1} *M. butyricum*; Difco) or ICFA (Difco) on day 0, followed on day 21 by an intraperitoneal injection of 100 μg of the same collagen type dissolved in acetic acid. *a*, Onset and incidence of arthritis in male mice given type II collagen in CFA (●); type II collagen in ICFA (○); type I collagen in CFA (◇). *b*, Onset and incidence of arthritis in female mice given type II collagen in CFA (▲); type II collagen in ICFA (△), type I collagen in CFA (□).

examination. Several joints of a single animal were often involved, sometimes so severely that the mouse had to be killed. The onset of arthritis in female mice injected with type II collagen in ICFA was delayed—swelling was never seen before day 36 in any joint. The incidence of the disease was lower in male and female mice injected with type II collagen in IFCA (67%) than in animals receiving type II collagen in CFA (100%).

The incidence and day of onset of arthritis in mice injected in four separate experiments are shown in Fig. 1. Of 40 mice injected with type II collagen in CFA, all developed arthritis, compared with 18 of 27 injected with type II collagen in ICFA. None of the 12 mice injected with type I collagen in CFA, or of the 17 mice injected with acetic acid and CFA, showed signs of the disease (Table 1).

Histological examination of affected joints from animals receiving type II collagen either in CFA or ICFA revealed initial oedema of the synovium and perisynovial tissues, followed by synovial hyperplasia. There was an early massive, predominantly polymorph and subsequent mononuclear cell infiltration into the synovium, sub-synovium, joint cavity and periarticular tissue. This was followed by pannus development, fibrin deposition, and cartilage and bone erosion with new bone formation. Late in the course of the disease the cellular infiltration and oedema were reduced, but by then many joints had become ankylosed, and most recognisable synovium and joint cartilage had been destroyed. The vertebrae were rarely affected, involvement occurring only several weeks after initial peripheral joint swelling. Very occasional, small inflammatory foci in the ear or tail were seen in a few animals.

Several important negative observations were made. There was no clinical or histological evidence of arthritis after injection of type I bovine collagen or acetic acid in CFA. Uninoculated control animals kept in the same cage as mice injected with type II collagen in CFA did not develop any clinical or postmortem evidence of arthritis. Synovial cavity washes of affected and unaffected joints from 17 DBA/1 mice were cultured for mycoplasma and all gave negative results. There was no significant growth of bacteria when synovial cavity washes from one unaffected and four affected animals were cultured anaerobically and aerobically, and no bacteria were seen in histological preparations of joints.

There are differences between the disease in the rat as described by Trentham *et al.*[1] and that seen in the mouse. Arthritis in the rat appeared earlier and it could be produced by immunisation with antigen in either CFA or ICFA. Mice responded less well to use of ICFA as adjuvant. A single intradermal injection has produced the disease in the rat, but only a schedule of initial intradermal immunisation followed by a subsequent challenge has been used in the mouse. We have found in both species, that involvement of second, third and fourth paws may occur up to 4 weeks after swelling of the first joint and that the number of joints affected varies between animals. The histology in the two species is similar.

Initial studies in other strains of mice treated in the same fashion showed that arthritis may sometimes be produced, but its clinical and histological incidence was variable, and the severity has been less than in the DBA/1 mice: SWR (major histocompatibility type H-2q)—4/15 (total number with clinical or histological lesions/total number treated); SJL (H-2s)—4/6, BALB/c (H-2d)—4/27; BlO.G (H-2q)—4/11; DBA/1 mice from the Jackson Laboratory, Bar Harbour, Maine—2/5 (the Carshalton colony was derived from this colony 25 years ago (M. W. Festing, personal communication); male and female mice from both colonies are histocompatible on skin grafting and *in vitro* mixed lymphocyte culture (L. Berumen, personal communication). Groups of 5–10 mice of the following strains did not develop any joint lesion after treatment with collagen type I or II in CFA or ICFA—C57Bl/10scSn (H-2b), CBA/CA (H-2k), BlO.A (5R) (H-2i5), BlOBR (H-2k) and DBA/2 (H-2d).

It is known that the immune response to the native helical portion of the bovine type II collagen molecule differs in strains of inbred mice[3] and that the response appears to be under H-2 linked control[4]. Trentham *et al.*[5] have shown that in rats the conformational antigenic site on the collagen molecule is important in induction of arthritis, since isolated α-chains of type II collagen did not produce arthritis when injected intradermally in either CFA or ICFA. Initial work has been undertaken here to study the anti-type II collagen antibody response in susceptible and resistant strains of mice, the role of the histocompatibility type and to examine the immunological processes involved.

Table 1 The incidence of arthritis induced by bovine collagen type II in DBA/1 mice

	Bovine collagen type given on day 0 and on day 21	Adjuvant given on day 0	No. of mice affected by day 62/total mice given treatment
Male	II	CFA	24/24
	—	CFA	0/10
	—	-	0/5*
	II	ICFA	12/15
	—	ICFA	0/4
	I	CFA	0/5
Female	II	CFA	16/16
	—	CFA	0/7
	II	ICFA	6/12
	I	CFA	0/7

* Kept in same cage as affected animals.

The finding that an arthritis develops in mice after immunisation with type II collagen lends some support to the hypothesis of Trentham *et al.*[1] that an immune reaction to collagen may be involved in the pathogenesis of arthritis. Use of the mouse disease as a model should avoid the risk of adjuvant arthritis common to experiments in the rat, and it should also permit ready analysis of the role of histocompatibility type and immune response genes.

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Human antibody reacts with a B-cell subset in man to induce B-cell differentiation

Jean Claude Brouet, Catherine Grillot-Courvalin, & Maxime Seligmann

Laboratory of Immunochemistry and Immunopathology
(INSERM U108, Research Institute on Blood Diseases and
Laboratory of Oncology and Immunohematology of CNRS),
Hospital Saint-Louis, 75475 Paris, Cedex 10, France

The events leading to B-cell differentiation and maturation into plasma cells in man are still poorly defined. Human B-cell differentiation has mainly been studied after triggering by nonspecific mitogens which react with unknown B-cell-surface structures. T cells and T-cell factors play an important part in this B-cell activation[1]. It is therefore interesting that we found that some patients with the rare immunodeficiency disease, the Wiskott-Aldrich (WA) syndrome, possess an IgM antibody which reacts only with a subset of human B-cells from the peripheral blood and tonsils and which is able to induce B cells to differentiate into plasma cells *in vitro*. This triggering is largely independent of T cells and in certain conditions occurs in the absence of detectable B-cell proliferation. In view of certain recent findings in some immunologically defective strains of mice such as CBA/N, genetic immunodeficiencies in man may represent useful models for investigating human B-cell subsets and the process of B-cell activation. We have therefore looked for B-cell antibodies in the serum of WA patients because, in addition to an immune defect of poorly understood mechanism[2], these patients have major thrombocytopenia and are therefore multi-transfused. We found that the serum of seven of nine WA patients contained an antibody reacting by cytotoxicity and immunofluorescence tests with a subset of both normal human and, unexpectedly, of the patients' own B cells.

A two-step double labelling procedure was used for the immunofluorescence experiments. In the first step, membrane-bound B-cell immunoglobulins (Ig) were redistributed with fluoresceinated IgG or F(ab')$_2$ fragments from antibodies to human Ig (or μ chains), allowing the enumeration and identification of B cells. In the second step, the cells were incubated in non-capping conditions with heat-inactivated sera of the various patients or controls, washed and finally stained with rhodamine-conjugated F(ab')$_2$ fragments of monospecific rabbit IgG antibodies to human Ig chains[3]. Whereas the control sera, including 15 patients with varied immunodeficiencies, did not stain a sizeable population of B cells, the serum of seven WA patients reacted with 30–60% of B cells from human tonsils and from the peripheral blood of all 30 normal donors tested. The antibody belongs to the IgM class and is polyclonal because the 19S (and not 7S) fraction obtained by preparative ultracentrifugation of the serum in a sucrose density gradient was reactive and, in immunofluorescence tests staining was observed only using F(ab')$_2$ fragments of antibodies to μ, κ and λ chains (and not to γ, α or δ). Most sera had a titre of 1/4 to 1/16 and reacted with B cells at both 4 °C and 37 °C. Two sera had an end point of 1/32 and 1/128 respectively and were also studied by cytotoxicity at 25 °C on purified B- and T-cell populations from selected donors with various HLA phenotypes. These experiments confirmed that the antibodies were unreactive with T cells and killed a subset of B cells.

The reactive antigenic determinant on the surface of B cells is independent of surface Ig (and specially IgD) as shown by double-labelling and redistribution experiments. Double labelling experiments also showed that the reactive structure is unrelated to Ia-like (or Dr) antigens, as could be expected in view of its presence on only a B-cell subset. For the same reason it seems very unlikely that the WA antibody reacts with other well defined B-cell-surface molecules such as the receptors of IgG, IgM, complement components, or Epstein–Barr virus. In addition it should be recalled that the antibody was unreactive with T cells and monocytes, as well as red cells and platelets.

The antigenic structure is presumably a marker of a unique subset of B cells and does not seem to be a differentiation antigen since we found that it is expressed on pre-B cells of a patient with pre-B-cell acute lymphoblastic leukaemia, on all leukaemic cells from two of five patients with B-derived chronic lymphocytic leukaemia and on the plasma cells from two of four myeloma cases. Experiments to define the biochemical properties and molecular weight of this antigenic structure are in progress. It is probably distinct from the glycoprotein of molecular weight 54,000 (gp 54), recognised by a rabbit antiserum and recently described by Wang *et al.*[4], which is also a marker of a subset of human B cells, since interaction of these surface molecules with their specific antibodies mediates different functional effects.

Whereas antibodies to the gp 54 antigen stimulated B lymphocytes to proliferate but not to differentiate into plasma cells, we found that the WA antibody does induce B-cell maturation. Table 1 shows that when peripheral blood lymphocytes were cultured in the presence of 1% WA serum (or its 19S fraction at a concentration of 10 μg ml^{-1}), a 5- to 20-fold increase in the percentage of plasma cells above control cultures was found on day 7, together with a striking cell proliferation and increase in surface Ig-positive cells. The pattern which was

Table 1 *In vitro* induction of B-cell differentiation by WA serum

		Cytoplasmic Ig*			Membrane Ig*			
		μ	γ	α	δ	μ	γ	α
Expt 1	WA serum	12	8	5	0	23	5	8
	NHS	1.5	0.5	<0.5	0	7	5	3
	PWM	15	13	11	0	18	3	9
Expt 2	WA serum	15	6	3	0	22	2	5
	NHS	1	0.5	<0.5	0	6	4	2
	PWM	8	12	11	0	12	2	10
Expt 3	WA serum	14	6	7	0	21	3	6
	NHS	0.5	1	0	0	8	4	2

Mononuclear cells from peripheral blood were isolated by Ficoll–Hypaque gradient. The cells were washed three times in RPMI medium 1640 containing 10% FCS and antibiotics. 5×10^6 cells were incubated for 7 days in a 5% CO_2/95% air incubator in 5 ml of the same medium containing 1% WA serum, 1% normal human serum (NHS) or 50 μg ml^{-1} PWM. On day 7, cells were washed and studied for surface and cytoplasmic Ig by direct immunofluorescence with monospecific antisera to human Ig heavy chains.

* Percentage of positive cells.

Table 2 *In vitro* induction of B-cell differentiation by WA serum using B-cell enriched or unseparated PBL and two different batches of FCS

		Unseparated PBL			E-rosette-depleted PBL		
		WA serum	NHS	PWM	WA serum	NHS	PWM
Expt 1	cIg*	27	1.5	21	5	0.5	2.5
Expt 2	cIg*	1	<0.2	8	2	<0.2	<0.2
	SIg*	10	3	15	67	20	34
Expt 3	cIg*	1.5	<0.1	10	3.5	<0.2	0.5
	SIg*	34	7	17	66	18	40

Unseparated peripheral blood lymphocytes (PBL) or B-cell enriched populations obtained by Ficoll–Hypaque gradient separation of lymphocytes rosetted with neuraminidase-treated sheep erythrocytes (two successive separations) were cultured for 7 days with 1% WA serum, 1% normal human serum (NHS) or PWM as described in Table 1. Experiment 1 was performed with the batch of FCS used in Table 1 whereas experiments 2 and 3 were performed with another batch of FCS (see text).

* Percentage of positive cells.

observed was very close to that of pokeweed mitogen (PWM)-induced B-cell differentiation and the isotype distribution of intracytoplasmic Ig showed a slight predominance of IgM over IgG and IgA plasma cells. These figures were obtained with a given batch of fetal calf serum (FCS). When using another batch of FCS, there was still a 5- to 10-fold increase in the percentage of Ig-bearing immunoblasts and of plasma cells in the presence of the WA antibody as compared with normal serum. However, in these conditions, the number of plasma cells in the culture was smaller and the differentiation of B lymphocytes occurred without detectable cell proliferation, as assessed by the absence of thymidine uptake on any day during the period of culture. The discrepancy between these two sets of results using different batches of FCS was explained by the study of the effect of the WA antibodies on purified B cells. When using such purified B-cell preparations, the cell recovery and plasma cell number were similar for both batches of FCS (Table 2). We therefore conclude that B-cell differentiation induced by the WA antibody can occur after depletion of T cells. T cells are needed to obtain B-cell proliferation and optimal differentiation; this T-cell effect requires the presence of a supportive batch of FCS.

Since PWM probably only acts on a given B-cell population[1], experiments were designed to investigate possible relationships between the B-cell subset reacting with the WA antibody and that responsive to PWM. There was no additive effect when lymphocytes were cultured in the presence of both PWM and WA serum. On the other hand, the depletion by cytotoxicity of those B cells reactive with the WA antibody resulted in a 60–80% decrease in the number of plasma cells obtained in PWM-stimulated cultures. These results show that the two B-cell subsets are similar or at least overlapping. Since PWM-induced B-cell differentiation is mediated by T-cell factors, it is worth investigating possible interactions between the WA antibody and a receptor for T-cell helper factors.

These experiments demonstrate that the antibodies to a subset of B cells found in the serum of some patients with the WA syndrome interact with a surface molecule involved in B-cell triggering. To our knowledge, such antibodies have not previously been described in man. Their properties are somewhat reminiscent of those of antibodies directed to the receptor for lipopolysaccharide on mouse B cells[5] and of anti-Ly 3 antibodies which markedly enhance the development of antigen-specific plaque-forming cells[6]. The surface antigen defined by our antibody may be similar to the murine Ly 3 antigen which is a marker of the B-cell subset absent in CBA/N mice[6]. The availability of the human antibody described here may help to characterise such human lymphocyte markers and to obtain insights into the control of human B-cell activation.

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Osteoclasts derived from haematopoietic stem cells

Patricia Ash, J. F. Loutit & K. M. S. Townsend

Medical Research Council, Radiobiology Unit,
Harwell, Didcot, Oxon OX11 0RD, UK

The origins of the multinucleated osteoclast have been controversial, with osteogenic precursors[1] and haematopoietic stem cells[2] as candidates. Recent evidence for the latter is persuasive but circumstantial. We report here direct evidence obtained in radiation chimaeras from a natural cytoplasmic cell marker transmitted by the donated haematopoietic stem cell.

Beige mice (Chediak-Higashi syndrome) have giant lysosomes, present in granular leukocytes and monocytes[3] and osteoclasts[4]. Lethally X-ray irradiated (600 rad) osteopetrotic weanling microphthalmic (*mi*/*mi*) mice have been converted to radiation chimaeras[5], by intravenous infusion of bone marrow from beige (*bg*/*bg*) mice. This gives complete regeneration of the lymphomyeloid complex from donor's haematopoietic stem cells, but osteogenic cells and their precursors remain of host-type[6,7]. In the course of restoration, the osteopetrosis, attributed to a genetic defect of osteoclastic mechanisms, is cured[8,9], the process being evident after 2 weeks, advanced at 4 weeks and complete by 6 weeks.

Table 1 shows that the per cent area of giant lysosomal granules in osteoclasts observed electron micrographically increases from a baseline of near zero to 0.15% at 2–4 weeks, a difference statistically significant ($P < 0.001$) but not impressive to a visual observer; at 6 weeks this is nearly 2%, the figure observed for beige mice[4]. This level persists until the last observations, made after 4 months; that is, the osteoclasts of the chimaera, like the granular cells, notably eosinophils, exhibit the characteristics of the beige mouse donor. Osteoblasts remained without giant lysosomes.

Table 1 The per cent area of osteoclasts occupied by giant lysosomes ($>1\ \mu$m diameter)

Group	Days after transplant (no. in groups)	Mean % of osteoclast area due to giant lysosomes	
Experimental	14 (1)	0.18	$\bar{x} = 0.15 \pm 0.004$
	28 (3)	0.12	
	42 (3)	1.84	$\bar{x} = 1.89 \pm 0.35$
	56 (3)	1.87	
	120 (2)	1.99_5	
Control CBA	— (5)	0.02	$\bar{x} = 0.018 \pm 0.008$
Control *mi*/*mi*	— (5)	0.016	
Control *bg*/*bg*	— (5)	2.09 ± 0.58	

For each animal, 30–40 photographs of osteoclasts, observed electron micrographically in metaphyses of femora and ilia, were taken at a magnification of ×3,000, two to five pictures being required to cover what seemed to be a single osteoclast. The photographic negatives were analysed on a Quantimet (Cambridge Instruments). The measurements of area in picture points were converted to μm^2. A computer program was written to coordinate osteoclast area and number of, and area occupied by, giant lysosomes.

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We conclude that the ultimate osteoclasts of the chimaera derive from the haematopoietic stem cells of the beige mouse donor. During the first 2–4 weeks, resolution of the osteopetrosis proceeded steadily. Then, the osteoclasts with brush borders observed electron micrographically, although not yet formally enumerated, became more common than in normal mouse bone. However, the emergence of the lysosomal marker in these cells was variable, observable but not impressive. There may be several explanations for this: (1) only a minor population of donor's osteoclasts may be adequate for the job of clearing the redundant bone. (2) Osteoclasis was being pushed to its limit. Osteoclasts working overtime may not form giant lysosomes by fusion of small lysosomes. (3) Osteoclasis of this degree may be a function of uninucleated precursor cells[10,11] not scored in our investigation. Other elements of the mononuclear phagocyte system, such as tissue phagocytes[2], have been invoked in osteoclasis, and blood monocytes are reported to be lytic for dead bone[12,13]. (4) The graft of myeloid tissue confirmed in other investigations[14,15] by the nuclear marker (T6 chromosome) may have the additional ability to repair the defect of the host's osteoclasts by short-range exchange of 'information', that is, plesiopathy (πλησίος, near; πάθος, experience) as between lymphocytes and cells of the mononuclear phagocyte system.

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Pacemaker currents in chick embryonic heart cells change with development

Alvin Shrier* & John R. Clay†

Department of Anatomy, Emory University, Atlanta, Georgia 30322

The initial heartbeat of the chick embryo occurs shortly after the first day of incubation[1,2]. The pacemaker of this beat originates in the region of the primitive heart destined to become sino-atrial tissue in the adult[3]. Individual cells isolated from the atrial and ventricular portions of the embryonic heart are also capable of beating spontaneously at this stage[4,5]. However, the intrinsic activity of these cells gradually diminishes from about day 7 until day 21 when the chick hatches[5]. We have investigated these changes in automaticity by measuring membrane pacemaker currents with the voltage-clamp technique from aggregates of cells prepared from 7-, 12- and 17-day-old chick embryo cardiac ventricle. We report that there is a voltage and time dependent conductance at 7 days which is markedly reduced by 17 days. The reduction of this pacemaker current parallels the decrease of spontaneous activity in these preparations.

Spheroidal heart cell aggregates (HCA) were prepared using techniques previously described[6]. Briefly, the apical portions of heart ventricles were dissected from chick embryos incubated for 7, 12 or 17 days at 37 °C. The tissue was dissociated into its component cells in 0.05% trypsin (1:300 Nutritional Biochemical) by a multiple cycle dissociation process[7]. Cells were allowed to reaggregate in a flask containing tissue culture medium which was placed on a gyratory shaker for 48–72 h. During gyration HCA were formed which were typically from 100 to 300 μm in diameter (D). The HCA were allowed to adhere to the bottom of a tissue culture dish containing medium 818A (ref. 6). Potassium concentrations (K_o) used ranged between 1.3 and 6 mM. The temperature of the dish was maintained at 37 °C (continuously monitored). Intracellular electrical recordings were made with glass microelectrodes filled with 3M KCl (resistance 15–50 MΩ). Measurements from all three developmental stages were made in the presence of 3 μM tetrodotoxin (Sigma) which acted to block the fast inward sodium current which seems to be fully developed before day 7 (ref. 8). Voltage-clamp measurements were performed with standard circuitry[9] on aggregates with $D = 180$–220 μm. Current was recorded from the voltage drop across a 10-MΩ feedback resistor of an operational amplifier (Analog Devices, Model 48K) which held the bathing medium at virtual ground via an agar salt bridge and an Ag:AgCl wire. The HCA is suitable for a voltage clamp study of pacemaker currents because: (1) the cells are well-coupled[10,11]; (2) the specific membrane resistance is large for pacemaker potentials[12]; (3) most of the cells are in the two or three outermost layers of the preparation because of its spherical geometry; and (4) the extracellular cleft spaces are relatively large[12,13].

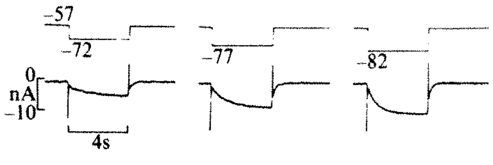

Fig. 1 Voltage clamp records from 7 day HCA in 1.3 mM K_o. Rise time of voltage control was 5–15 ms. Current records were filtered with a low pass filter having 3 dB cutoff at 50 Hz.

The responses to rectangular voltage clamp steps at 7 days in 1.3 mM K_o are shown in Fig. 1. These steps produced a time-

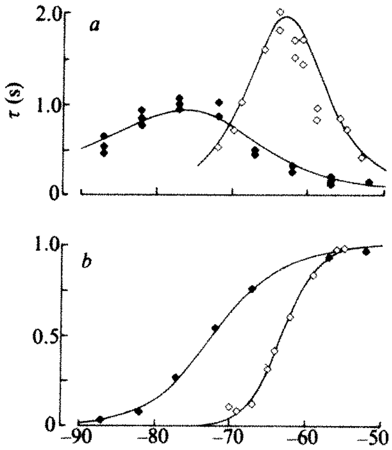

Fig. 2 Analysis of time-dependent currents in 7 (◆) and 12 (◇) day HCA. The currents from all developmental stages could be described by a single exponential function with time constant τ. For each pulse record τ was determined from a photograph or an oscilloscope trace using a Hewlett-Packard 9810A calculator, 9864A digitiser, and 9862A plotter. Solid lines are theoretical best fits to each set of data using modified forms of the model of pacemaker current in cardiac Purkinje fibre of McAllister *et al.*[17]. *a*, Voltage dependence of the time constant (τ) obtained from a single 7 or 12 day HCA pooled in each case from 4 different holding potentials. *b*, Normalized current amplitude on return to a single holding potential obtained by the method in ref. 14.

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* Present address: Department of Physiology, McGill University, Montreal, Quebec, H3G 1Y6, Canada.

† Present address: Marine Biological Laboratory, Woods Hole, Massachusetts 02543.

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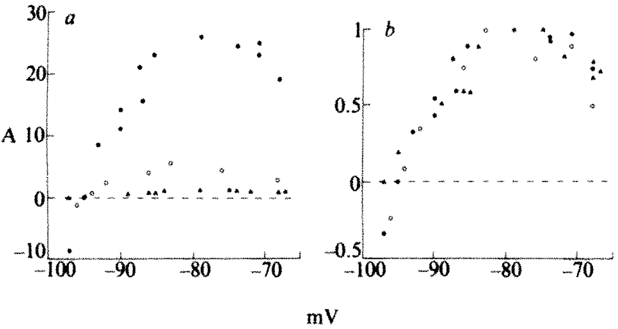

Fig. 3 *a*, Pacemaker channel current–voltage relationship[14] for 7 (●), 12 (○) and 17 day (▲) HCA in 3.5 mM K_o. The points were from a single preparation at each developmental stage. *b*, Normalised channel current–voltage relationships obtained by dividing each set of points in *a* by the corresponding peak current (I_p).

dependent change in membrane current which reached a steady final level corresponding to the voltage to which the membrane was clamped during the step. Successively smaller time-dependent currents were observed at 12 and 17 days, respectively, in the −55 to −120 mV range of membrane potentials. The current changes in this voltage range obeyed first order kinetics amenable to standard Hodgkin-Huxley analysis[14–16] (Fig. 2). The maximum time constant was 1 s at 7 days compared to 2 s at 12 days (Fig. 2*a*), and the range of activation of the kinetics was narrower at 12 days than at 7 days (Fig. 2*b*). The voltage at which the maximum time constant occurred at 12 days appeared to be shifted in the positive direction. The kinetics and the voltage at which the maximum time constant occurred at 17 days were both approximately the same as those at 12 days. Changes of K_o in the 1.3 to 6 mM range did not alter the voltage range or the time scale of the kinetics.

The amplitude of this pacemaker current may be ascribed to ion flow through membrane channels, each of which is gated by a particle having kinetics as described in Fig. 2. The voltage dependence of the channel current may be determined from the ratio of the time-dependent current change during the clamp step to the time-dependent current change after the voltage returns to the holding potential[14]. The results of this analysis for the three developmental stages in K_o of 3.5 mM are shown in Fig. 3*a*. The current inwardly rectifies and has a negative slope region positive to −80 mV. The relative voltage dependence of the channel current was nearly the same at all three stages (Fig. 3*b*). The peak channel current (I_p) was approximately independent of K_o, although the voltage at which I_p occurred was shifted by K_o and the reversal potential of the channel current changed with K_o like a potassium electrode. The pooled results for I_p were 27.2 ± 2.6 nA (±s.d., $n = 7$) at 7 days, 3.4 ± 1.1 nA ($n = 5$) at 12 days and 0.8 ± 0.4 ($n = 5$) at 17days. The reduction of this current could be produced either by a reduction of the channel density or by a reduction of the single channel conductance.

This study is the first analysis of the changes occurring in the ionic pacemaker currents of heart muscle cells during embryonic development. The contribution of the pacemaker current we have described to the control of beat frequency requires analysis of the time-independent current in the pacemaker voltage range, which also changes during development. This current becomes less inward with development and seems to be the dominant factor responsible for the loss of automaticity in these preparations.

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PGE_2 stimulates gastric chloride transport: possible key to cytoprotection

Rudolf Schiessel, Jeffrey Matthews, Ami Barzilai, Aryeh Merhav & William Silen

Departments of Surgery, Harvard Medical School, Beth Israel Hospital, 330 Brookline Avenue, Boston, Massachusetts 02215

Prostaglandins protect the stomach against a variety of noxious agents independently of effects on acid secretion, but the mechanism of this 'cytoprotection' is unknown[1]. We recently proposed[2] that gastric surface cells extrude or eliminate luminal acid by a process analogous to that described in squid axon[3], snail neurone[4], and barnacle muscle[5]. Influxing luminal H^+ combines with HCO_3^- which has entered the cell in exchange for intracellular chloride, probably at the nutrient membrane. Dehydration of the resulting H_2CO_3 into CO_2 and H_2O is catalysed by carbonic anhydrase, which is present in surface cells in large amounts[6]. Interference with this chain of reactions at any point frequently causes ulceration[2]. We have examined the effect of 16,16-dimethylprostaglandin E_2 (PGE) on different segments of this protective mechanism and show here that the protective effects are intimately associated with stimulation of chloride transport. All experiments were done *in vitro* thus eliminating any effects of prostaglandin on mucosal circulation.

Fundic or antral mucosa of bullfrogs (*Rana catesbeiana*) were mounted in lucite chambers after the frog was pithed and the muscular coat stripped from the mucosa[2]. The flux of $^{36}Cl^-$ was measured from nutrient (N) to secretory (S) solutions ($J_{Cl_{ns}}$), and vice versa ($J_{Cl_{sn}}$) in paired (fundus) and unpaired (antrum) tissues while the potential difference (p.d.), resistance (R) and short-circuit current (I_{sc}) were recorded[7]. Under standard open circuit conditions 10^{-5} M PGE caused a transient decrease followed at 60 min by a significant increase in $J_{Cl_{ns}}$. A concomitant rise in $J_{Cl_{sn}}$ and I_{sc} was observed. The increase in I_{sc} is probably caused by the fact that the inhibition of H^+ secretion is not accompanied by a decrease in the net transport of Cl^- (Fig. 1). Similar increases in unidirectional J_{Cl} were observed in short-circuited antrum except that the initial drop in $J_{Cl_{ns}}$ was not present (Fig. 2). The marked decrease in $J_{Cl_{ns}}$ and $J_{Cl_{sn}}$ noted when HCO_3^- is removed from the nutrient solution in metiamide-inhibited tissues was not influenced by the presence of 10^{-5} M PGE in N ($n = 4$). Addition of 4 mM SITS (4-acetamido-4′-isothiocyano-stilbene-2,2′ disulphonic acid) known to block anion transport in a variety of tissues, to N of fundic mucosa for 160 min decreased $J_{Cl_{ns}}$ from 9.49 ± 0.96 to 4.72 ± 1.03 μ Eq h^{-1} cm^{-2} and $J_{Cl_{sn}}$ from 5.64 ± 1.07 to 3.01 ± 0.44 μ Eq h^{-1} cm^{-2}, (mean ±s.e.; $n = 3$) and also reduced the p.d. and I_{sc} by 28.2% and 42.6% respectively. Histamine-stimulated H^+ secretion was decreased 49% by SITS. When 10^{-5} M PGE was added to fundic mucosae

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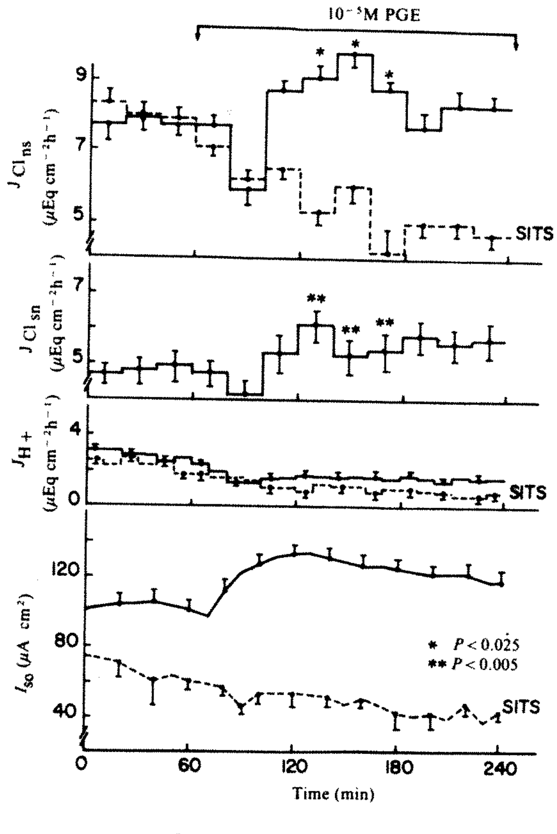

Fig. 1 Effect of 10^{-5} M PGE on Cl^- transport in fundus under open circuit conditions. The bathing solutions contained (in mM) 95 NaCl, 23 Na isethionate on the secretory side and 87 NaCl, 18 $NaHCO_3$, 4 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 1 KM_2PO_4 and 10 mM glucose on the nutrient side and were gassed with 95% O_2–5% CO_2. At 60 min after adding PGE, $J_{Cl_{ns}}$ ($n = 7$) and $J_{Cl_{sn}}$ ($n = 7$) increased significantly over the baseline values. This did not occur in 7 untreated controls. PGE inhibited H^+ secretion and increased p.d. from 28.6±2.53 to 38.4±1.73 mV (mean±s.e.) 60 min after application. R remained stable during this period (205±15.9 to 206±18.52 cm²). Pretreatment with SITS, 4×10^{-3} M for 60 min led to a decrease in $J_{Cl_{ns}}$ ($n = 6$) which was not influenced by PGE. Data from baseline and test periods were evaluated by Duncan's test. Comparisons between the means of these two periods were made by the t-test for paired values.

Table 1 Ulceration of gastric sacs

Buffer	Gas	n	Ulcerated	P
Control				
5 mM PO_4^{3-}	100% O_2	16	14	0.004
PG-pretreatment				
5 mM PO_4^{3-}	100% O_2	16	6	NS
5 mM PO_4^{3-} (Cl^--free)	100% O_2	16	11	
Control				
SITS+5 mM PO_4^{3-}	100%	8	8	
SITS+18 mM HCO_3^-	95% O_2–5% CO_2	8	4	
PG-pretreatment				
SITS+18 mM HCO_3^-	95% O_2–5% CO_2	8	4	
SITS+5 mM PO_4^{3-}	100% O_2	8	7	

Tied sacs of inverted gastric mucosa (mucosa inside) were incubated in different nutrient buffers circulated by the indicated gas. After 1 ml of artificial gastric juice, containing 40 mg % pepsin and 20 mM HCl, had been instilled into the sac lumen, the tissue was incubated for 4 hours. PG-pretreatment means addition of 3×10^{-6} M PGE 60 min before instillation of artificial gastric juice. The Fisher's exact test was used for statistical evaluation. NS, not significant.

pretreated with SITS, 4 mM no effect on $J_{Cl_{ns}}$ was observed (Fig. 1).

Forte[7] has described three components of $J_{Cl_{ns}}$ in amphibian gastric mucosa. In addition to the component of active transport dependent on the presence of O_2 and to a small diffusional component, a major fraction of $J_{Cl_{ns}}$ is represented by exchange diffusion dependent on the presence of Cl^- in the secretory solution. Since we postulate that the availability and exchange of cellular Cl^- for nutrient HCO_3^- are vital for protection of surface cells against luminal H^+, we tested the effect of PGE on exchange diffusion according to Forte[7]. When Cl^- was removed from the secretory solution, PGE was unable to maintain a normal exchange diffusion of Cl^- from N to S (Fig. 3), indicating that the increased Cl^- fluxes across the tissues induced by PGE are more likely a result of increases in exchange of HCO_3^- for Cl^-, than a direct stimulation of the Cl^- pump.

Prostaglandins stimulate an adenylate cyclase in surface cells but not the histamine-stimulated cyclase of oxyntic cells[8]. We added 10^{-3} M N^2-O^6-dibutyryl cyclic AMP to N, of short-circuited antral mucosa and found that $J_{Cl_{ns}}$ increased markedly within 40 min (Fig. 2).

To test the relationship of these findings to the known cytoprotective effects of PGE, we studied tied sacs of fundic mucosa of the same species known to ulcerate almost invariably in the absence of nutrient HCO_3^- (refs 2, 9). Ulceration was prevented to a highly significant degree by 3×10^{-6} M PGE, in the absence of nutrient HCO_3^- but not when Cl^- was replaced by isethionate and SO_4^{2-} in the bathing solution (Table 1). In sacs pretreated with 4 mM SITS, PGE failed to prevent ulceration not only in the absence of nutrient HCO_3^- but even when 18 mM HCO_3^- was present in the nutrient solution (Table 1). The protection afforded by the presence of HCO_3^- in tissues exposed to SITS was not enhanced by PGE.

We conclude that PGE stimulates $J_{Cl_{ns}}$ and $J_{Cl_{sn}}$ in a process possibly involving cyclic AMP. The failure of PGE to preserve forward Cl^- flux in the absence of Cl^- in the secretory solution suggests that PGE stimulates the exchange of cellular Cl^- for nutrient HCO_3^-, a vital step in the protection of the surface cell against the hostile environment of luminal acid. The importance of the availability of nutrient HCO_3^- for such an exchange is emphasised by recent studies (in preparation) showing that

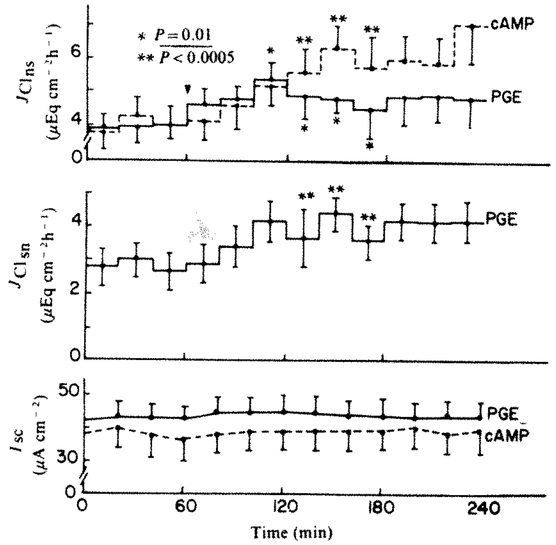

Fig. 2 Effect of 10^{-5} M PGE on Cl^- transport in antrum under short-circuit conditions. $J_{Cl_{ns}}$ ($n = 8$) and $J_{Cl_{sn}}$ ($n = 6$) increased significantly over the baseline period values. This did not occur in 8 untreated controls. Cyclic AMP 10^{-3} M also increased $J_{Cl_{ns}}$. Arrow indicates addition of drug to the nutrient solution. Data evaluated as for Fig. 1.

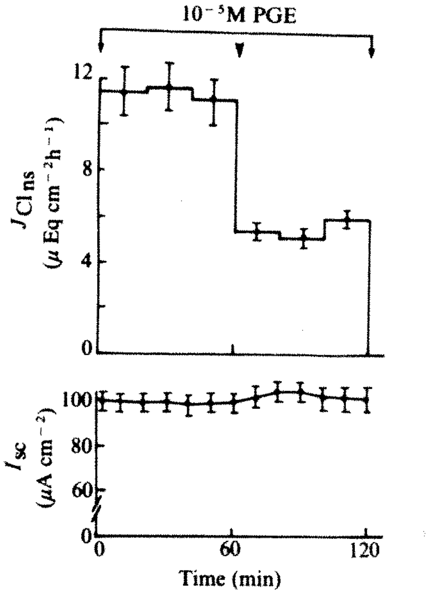

Fig. 3 Effect of 10^{-5}M PGE on the exchange diffusion component of $J_{\mathrm{Cl_{ns}}}$. At the arrow the secretory solution was replaced with 118 mM Na isethionate and 2 mM K_2SO_4. Duncan's test was used for statistical evaluation.

protection of the mucosa can be achieved with either a high concentration of nutrient HCO_3^- or by the actively stimulated oxyntic cell and its consequent alkaline tide. The protective mechanisms of the gastric epithelial surface cell bear many analogies to the acid extruding pump demonstrated in other tissues[3-5].

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Muscarinic suppression of a novel voltage-sensitive K^+ current in a vertebrate neurone

D. A. Brown* & P. R. Adams

Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550

Cholinergic excitation of vertebrate neurones is frequently mediated through the action of acetylcholine on muscarinic (atropine-sensitive) receptors. This type of excitation differs substantially from the better known nicotinic excitation. One difference is that, instead of an increased membrane conductance, a decreased conductance (to K^+ ions) frequently accompanies muscarinic depolarisation. This has been detected in sympathetic[1-4], cortical[5] and hippocampal[6] neurones. Using voltage-clamped frog sympathetic neurones we have now identified a distinctive voltage-sensitive K^+-current, separate from the delayed rectifier current, as the prime target for muscarinic agonists. We have termed this current the M-current, I_M.

For our experiments we used isolated lumbar sympathetic ganglia from bullfrogs (*Rana catesbiana*), from which the connective tissue was removed with trypsin (1% for 10–15 min): this treatment did not materially affect the currents under study. The ganglia were perfused with Ringer's solution at 22 °C containing 2.5 mM KCl, 2 mM $CaCl_2$, 2 mM Tris (buffered to *p*H 7.2 with HCl), and (usually) 10 mM $MgCl_2$ to improve electrode sealing. Individual cells were impaled with two microelectrodes filled with 3M KCl (resistances 20–50 MΩ), one for recording membrane potential and one for passing or recording current.

Some general properties of the M-current are illustrated in Fig. 1. This cell was held under voltage-clamp at a relatively depolarised membrane potential (−30 mV), at which there is normally a substantial and steady outward M-current. On applying a hyperpolarising step-command, the channels carrying the M-current gradually close, giving rise to a slow inward current relaxation after the initial fast ohmic step. Channel closure is indicated by the fact that the membrane conductance falls during this relaxation, the ohmic step at the end of the command being smaller than that at the beginning. Repolarisation then produces a slow outward relaxation as the M-channels open again. (On repolarising from membrane potentials more negative than −70 mV the currents are complicated by the appearance of another, more transient outward current analogous to the rapidly inactivating A-current reported in molluscan neurones[7,8].)

With increasing step-commands the inward relaxation reversed in direction at command potentials between −70 and −100 mV (−74 mV in Fig. 1). This reversal potential shifted in the depolarising direction by 20–25 mV on raising $[K^+]$out from 2.5 to 8 mM, and by 40–55 mV at 25 mM. Thus, I_M is largely, but perhaps not exclusively, a K^+ current.

The M-channels seem to be fully closed at membrane potentials more negative than −60 mV, as hyperpolarising commands from such potentials yielded only ohmic current steps. Above −60 mV the conductance attributable to the M-channels showed a sigmoidal increase, maximising at −10 to 20 mV (Fig. 1*c*). The position and total amplitude of the conductance–activation curve did not vary using different holding potentials between −20 and −50 mV, indicating that I_M does not show time-dependent inactivation within its activation range. The slope of the mean activation curves corresponded to an ε-fold shift in the equilibrium between open and closed M-channels for 6–7 mV depolarisation, as though the state of the channels was controlled by a tetravalent voltage-sensing particle moving through the entire membrane potential[9]. Both inward and outward M-current relaxations showed an approximately exponential time course (see Fig. 3), whose time constant diminished with hyperpolarisation from a maximal value of 150–200 ms at between −40 and −30 mV. These kinetic properties distinguish the M-current very clearly from the conventional delayed rectifier current[9]. Thus, in our bullfrog neurones, the activation threshold for the latter was about −25 to −20 mV, while the time constants for the repolarising tail-currents of the delayed rectifier were an order of magnitude faster than those for the M-current at comparable membrane potentials (see Fig. 4).

To test the effect of muscarinic receptor activation on the clamp currents we used (±)muscarine as the preferred agonist; this has minimal nicotinic activity on ganglion cells[10], thus obviating the need for nicotinic blockers. Under current-clamp (Fig. 2*a*), muscarine produced the characteristic slow depolarisation and conductance decrease previously described for other muscarinic agonists[1,4]. (In many cells muscarine also induced prolonged repetitive spike discharges following injection of current pulses.) Under voltage-clamp (Fig. 2*b*) the depolarisation was replaced by a steady inward current at depolarised membrane potentials, while the reduced conductance was manifest as a reduced current response to a hyperpolarising step-command.

Both the steady inward current and the reduced steady-state

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* Permanent address: Department of Pharmacology, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX.

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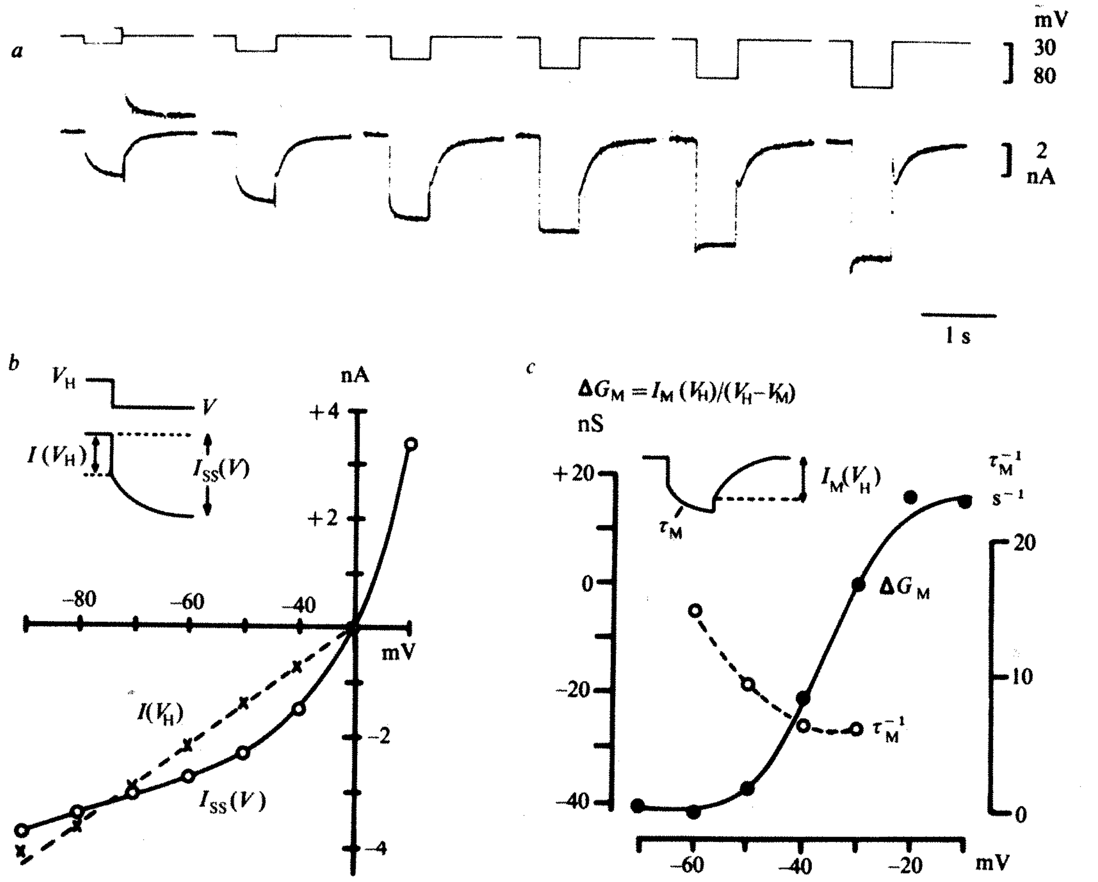

Fig. 1 M-currents in a voltage-clamped bullfrog neurone. The records in *a* show clamp-currents (bottom trace, inward current downwards) recorded during hyperpolarising step-commands from a holding potential (V_H) of -30 mV to command potentials (V) of -40 to -90 mV. The inverse current in the first panel is the slow tail of repolarising current after returning to V_H from a depolarising step-command to -20 mV. (This current record is shifted upwards for clarity; the outward current during the command is off-scale). In *b* the instantaneous (×--×) and steady-state (○——○) currents recorded in *a* are plotted against command potential (V). The instantaneous current is the amplitude of the fast transient $I(V_H)$ and the steady-state current is the total current measured after the slow relaxation is complete, $I_{SS}(V)$ (see diagram above). The intersection of the two gives the reversal potential (V_M) for the slow relaxation. The curves in *c* show the change of conductance attributable to closing and opening of the M-channels (ΔG_M, ●) and the reciprocal of the time constant of the slow relaxation (τ_M^{-1}, ○) plotted against command potential V. ΔG_M was calculated from the amplitudes of the M-current tails observed on repolarising from V to V_H (-30 mV), divided by the driving force V_H–V_M. (At $V = -20$, -10 mV only the slow component of the tail was measured, see Fig. 4*b*). τ_M Was calculated from semi-logarithmic plots of the slow inward relaxations on hyperpolarising from V_H to V (see Fig. 3 for examples). τ_M At -30 mV is the mean value for the relaxation observed on repolarising from V to $V_H = -30$, τ_M for inward and outward relaxation at the same final potential being equal.

conductance produced by muscarine result almost exclusively from a reduction of the M-current. Thus, since the M-channels would be closed at the end of the hyperpolarising command in Figure 2*b* ($V = -60$ mV, see Fig. 1*c*), the amplitude of the outward relaxation on repolarising the cell to -30 mV provides a measure of I_M at the holding potential of -30 mV. This repolarising M-current was depressed by an amount (2.2 nA) precisely equal to the magnitude of the steady inward current. Furthermore, muscarine produced negligible inward current at -60 mV. Direct proportionality between net inward current and I_M reduction throughout the potential range for I_M activation was confirmed in other experiments using different holding and command potentials. Finally, it may also be noted in Fig. 3 that the membrane conductance measured from the amplitude of the ohmic currents was greatly reduced at -30 mV but hardly at all at -60 mV.

From the time course of the M-current relaxations (Fig. 3) it appears that muscarine simply depressed the current amplitude

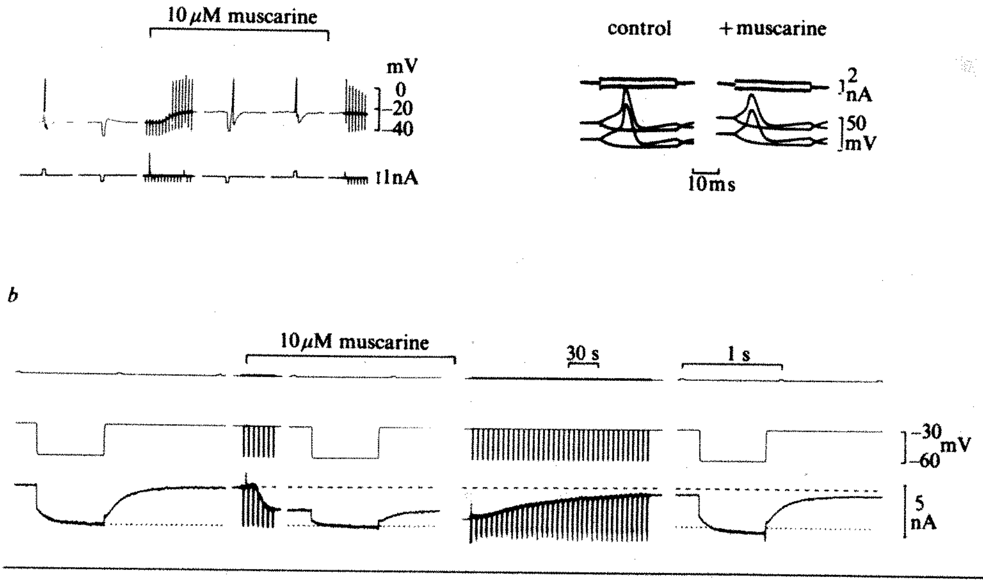

Fig. 2 Effects of muscarine on a single neurone recorded under: *a*, current-clamp, and *b*, voltage-clamp. The three traces on the chart-strip records read (from above downwards) time in s, membrane voltage, and current. The recorder was periodically accelerated to display the voltage and current transients. Muscarine was applied by rapid bath-perfusion. The oscilloscope records on the right side of the voltage response in *a* show superimposed spikes and electrotonic potentials evoked by depolarising and hyperpolarising current pulses respectively. The current pulses are on the top beam. Two voltage records are shown, the upper one being that recorded through the current-passing electrode via an active bridge circuit. In the voltage-clamp record (*b*, recorded earlier in the same cell), the membrane potential was held at -30 mV and commanded to -60 mV for 700 ms at 5-s intervals, and the induced currents recorded.

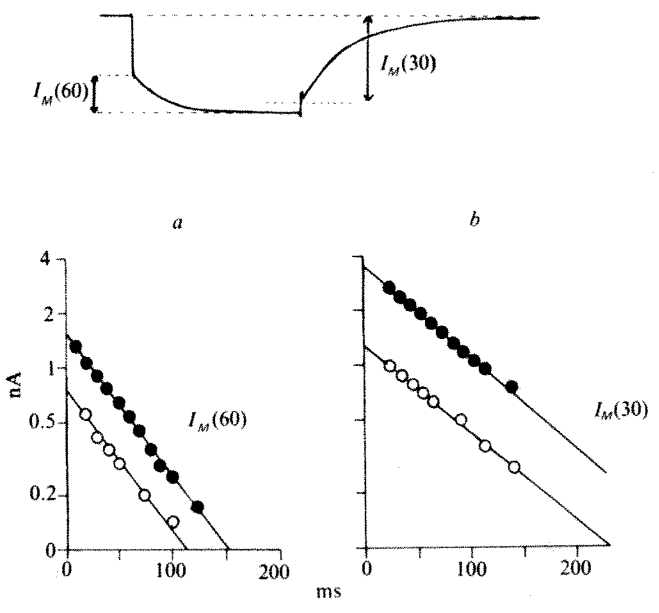

Fig. 3 Semi-logarithmic plots of the slow relaxations observed in Fig. 2*b* before (●) and during (○) muscarine application. *a*, Inward relaxation on hyperpolarising to −60 mV, I_M (60). *b*, Outward relaxation on repolarising to −30 mV, $I_M(30)$. The diagram above is a tracing of the first control current-relaxation in Fig. 2*b*, showing the measured currents.

without modifying its voltage sensitivity. Thus the ratio $\tau_M(30)/\tau_M(60)$ remained the same (1.5) in the presence and absence of muscarine. Hence any voltage sensitivity of muscarinic activation[3,11] appears to reside in the voltage dependence of the resting currents rather than in the action of muscarine *per se*. The effect of muscarine was concentration dependent, half the channels being shut at 1–3 μM, and was fully antagonised by 1 μM atropine. Varying $[Ca^{2+}]_{out}$ between 2 and 8 mM or $[Mg^{2+}]_{out}$ between 0 and 10 mM made no qualitative difference to the action of muscarine.

The unique action of muscarine on the M-current is best emphasised by comparing it with tetraethylammonium (Fig. 4): the latter profoundly depresses the delayed rectifier with little effect on the M-current, whereas muscarine suppresses I_M without depressing the delayed rectifier. (We did not see potentiation of muscarine-induced currents in 10 mM TEA solution; compare ref. 12.) The M-current was also unaffected by 1 mM Ni^{2+} which blocks inward Ca^{2+} currents in these neurones (data not shown here), or by 1–5 mM 4-aminopyridine, which inhibits the A-current of invertebrate neurones[8].

The function of the M-current is not yet clear. However, it does show some resemblance to the outward 'i_{slow}' of frog sinus tissue[13], and may likewise serve to limit firing frequency, by reducing the rate of depolarisation near the threshold for spike generation in the face of a steady or phasic inward current. The increased tendency toward repetitive spike discharges in cells treated with muscarine would accord with this: indeed, this, rather than the depolarisation itself may be the more significant feature of muscarinic excitation.

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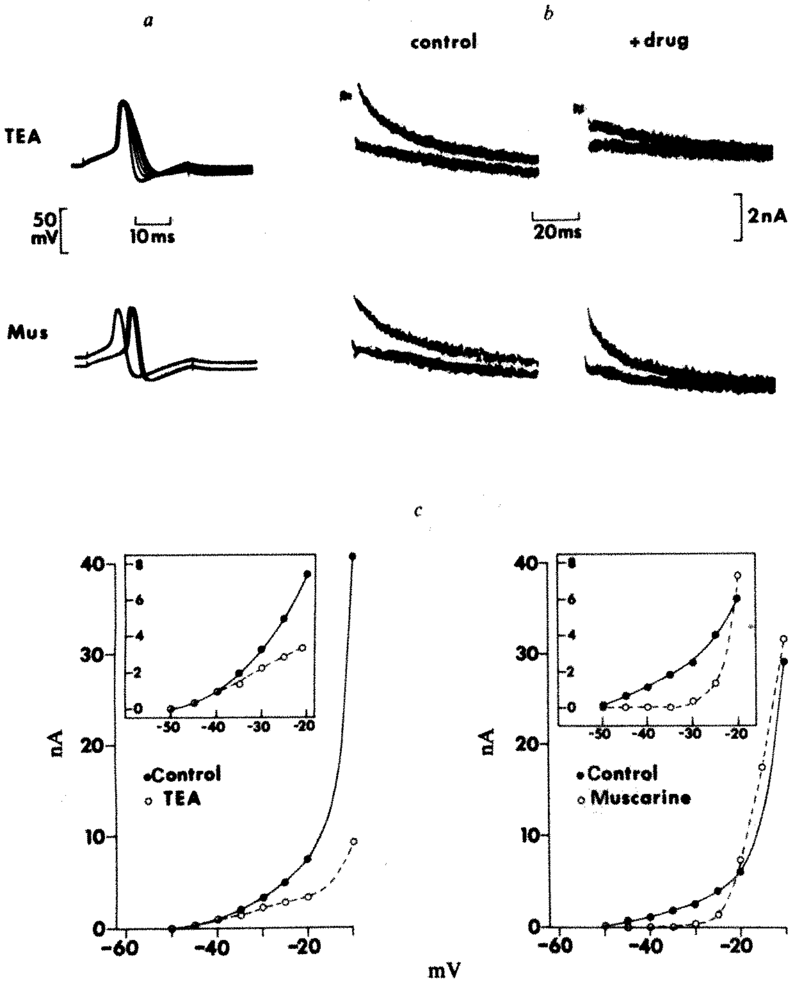

Fig. 4 Comparison of the actions of tetraethylammonium (TEA, 10 mM) and muscarine (Mus, 10μM) on *a*, unclamped action potentials, and *b* and *c*, repolarising current-tails and steady-state current–voltage curves respectively seen under voltage-clamp. The oscilloscope records in *a* show the action potentials evoked by injecting depolarising current through a single, bridge-balanced recording microelectrode, with control and drug-treated spikes superimposed. TEA prolonged spike-repolarisation without altering the membrane potential, whereas muscarine depolarised the cell without greatly affecting the spike. The records in *b* show the tail-currents seen on repolarising to the holding potential of −50 mV after depolarising commands to either −30 mV (lower trace) or −20 mV (upper trace). In the latter case, the tails showed two components, fast decaying and slow decaying, attributable to the closure of the delayed rectifier and M-channels respectively. TEA (upper records) suppressed the delayed rectifier tails; muscarine (lower records) did not affect the fast, delayed rectifier tail but reduced the amplitude of the slower M-current tail. (The apparent acceleration of the fast tail in muscarine solution is due to the loss of the slow tail.) The graphs in *c* show the steady state current–voltage curves from a single cell exposed first to TEA and then, after washing, to muscarine. The voltage-scale shows the pulse-potential from a holding potential of −50 mV. Current values (ordinates) are the steady currents attained at each pulse-potential minus the leak currents, extrapolated from the ohmic currents following step hyperpolarisations to −60 and −70 mV. The inset curves show the currents up to −20 mV on a larger scale. Note that TEA profoundly depressed the currents at membrane potentials more positive than −30 mV, whereas muscarine selectively depressed the currents below −20 mV.

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Somatostatin production by rat cerebral neurones in dissociated cell culture

J. Delfs*, R. Robbins†, J. L. Connolly‡, M. Dichter* & S. Reichlin†

* Departments of Neurology, Harvard Medical School and Beth Israel Hospital Division of Neuroscience, Children's Hospital Medical Center, Boston

† Division of Endrocrinology, Department of Medicine, Tufts-New England Medical Center, Boston

‡ Department of Pathology, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02115

Somatostatin (somatotropin release inhibiting factor, SRIF) is the 14-amino acid polypeptide found to be the active growth hormone-release inhibiting factor in extracts of sheep hypothalamus[1,2]. Although found in highest concentrations in the hypothalamus, it is widely distributed in the gastrointestinal tract and extra-hypothalamic nervous system. In fact, the cerebral cortex contains a larger total amount of somatostatin than any other brain region or visceral organ[3]. The source of somatostatin in extra-hypothalamic brain has been unclear. Initially, the hypothalamus was considered the only source, but elegant immunohistochemical demonstrations of positively staining neuronal perikarya in amygdala, hippocampus and neocortex suggested that these areas might be independent sources of somatostatin[4]. In addition, bilateral anterior hypothalamic periventricular lesions reduced somatostatin content in the median eminence to less than 10% of control levels, but did not affect its concentration in the preoptic area, amygdala or cortex[5]. Our laboratory has previously shown that dissociated rat cortical neurones in culture exhibit structural and electrophysiological properties similar to those of adult neurones *in situ*[6]. In conjunction with studies being carried out on the physiological effects of somatostatin on these neurones, we investigated the occurrence of endogeneous IR-somatostatin. We show here that dissociated cell cultures from rat cerebral hemispheres in the absence of any hypothalamic tissue produce immunoreactive (IR)-somatostatin. This IR-somatostatin is demonstrable histochemically in an exclusively neuronal population within the cultures.

Tissue was obtained by sterile removal of embryos from pregnant CD rats 15 d post-conception. The embryo skull was removed and slices were taken from antero-lateral cerebral cortex superficial to the lateral ventricles. The deeper portions of the cerebral hemisphere, including areas later giving rise to striatal structures, were discarded, as were the thalamus, hypothalamus, brainstem and cerebellum. The tissue was then minced, digested in trypsin, triturated, filtered for cell clumps, plated and grown in a medium consisting of Eagle's minimal essential medium, 5% heat-inactivated rat serum, 100 mg % glucose, 2 mM glutamine, 20 U ml^{-1} penicillin and streptomycin. The medium was changed three times per week and cells kept in a 5% CO_2 incubator at 36–37 °C. The details of the culturing procedures were as described previously[6], except that dishes in the present study were coated with collagen and polylysine instead of a layer of non-neuronal cells. Approximately 250,000 cells were initially plated in each culture dish.

For somatostatin assay, the medium (approximately 1.2 ml) was removed from the culture 3 d after the previous medium change, added to 1 ml 1 M acetic acid and frozen. The remaining cells were rinsed with physiological saline and extracted into 1.5 ml 1 M acetic acid and frozen. At the time of assay, samples were heated in boiling water, sonicated, centrifuged, lyophilised and resuspended in phosphate buffer (0.05 M with 0.1% bovine serum albumin (BSA), 0.1% sodium azide, *p*H 7.4). Radioimmunoassay was carried out with a well-characterised assay system[3].

No IR-somatostatin was detected in the initial cell suspension, in control media samples or in early cultures, but was detected in both cell extracts and media by day 7 of incubation (Fig. 1). Between 7 and 14 d the content in the cellular fraction increased dramatically. Concentrations of IR-somatostatin increased steadily to a maximum at approximately 3 weeks when the cellular fraction contained an average 3,033 ± 7.5 pg per dish and the media contained 326 ± 75.8 pg per ml media. The slightly decreased levels at points around 4 weeks in culture were thought to correspond to declining neuronal survival.

Chromatographic characteristics of the IR-somatostatin of both the cell extracts and the media were determined using a Biogel P-4 column with an ammonium acetate buffer (*p*H 4.7). The IR-somatostatin from both the cell extracts and the media eluted identically with the synthetic cyclic somatostatin standard. In addition, 8% of the IR-somatostatin in the cell extract migrated more slowly in a separate peak corresponding to a larger form of IR-somatostatin.

Immunofluorescent staining was carried out on cells cultured for 17–33 d on collagen–polylysine-covered glass coverslips. The coverslips were rinsed in a phosphate-buffered saline (PBS) and fixed in 3% formaldehyde in PBS and finally in cold acetone. The acetone step was added to increase the permeability of the fixed cell membranes and resulted in a marked increase in specific immunofluorescence over that obtained with formaldehyde alone, but with preservation of cellular morphology.

After fixation, a rabbit antibody prepared against BSA-conjugated somatostatin was applied at a 1:200 dilution with

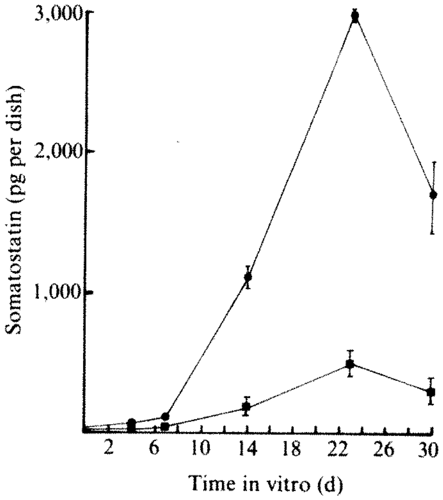

Fig. 1 Graph of IR-somatostatin in cell fraction (●) and in media fraction (■) per 35-mm dish containing dissociated cell culture of rat embryo cerebrum against days *in vitro* (mean ± 1 s.d. for determinations on three cultures). Total volume of cells is very small (<0.1 cm^3 per dish) compared with the volume of media (~1.2 cm^3 per dish), which emphasises the greater amount concentrated in cells. Levels of IR-somatostatin are undetectable until approximately day 7. Between days 7 and 23 the levels increase dramatically. The late fall-off probably indicates some deterioration in the cultures rather than a specific decline in somatostatin production.

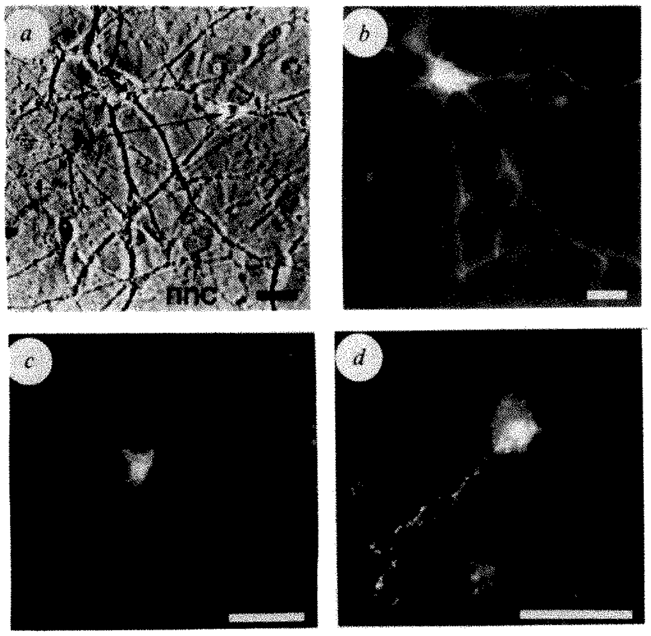

Fig. 2 Phase-contrast and indirect immunofluorescent microscopy of neurones in the dissociated cell cultures. *a*, Dispersed cerebral cell culture at 32 d seen under phase-contrast microscopy. Note flat non-neuronal cells (nnc) as well as neurone cell bodies (N) and neuronal processes. *b*, *c*, *d*, Fluorescence photomicrographs showing cultures stained with anti-somatostatin using indirect immunofluorescent technique. *b*, Same field as *a*, showing positive staining of the perikaryon and its neuronal processes. Note the selective staining of this neurone as distinguished from the several unstained neurone cell bodies and many unstained neuronal processes, prominently seen in *a* by phase contrast. *c*, Pyramidal-shaped neurone positively stained by anti-somatostatin in the presence of more dimly seen negatively staining neurones of roughly the same size in culture at 18 d. Staining in perikaryon of neurones often seemed to be continuous with staining in the proximal part of cell processes. *d*, Neurone positively stained by anti-somatostatin with smaller collections of staining trailing out a neuronal process in an 18-d culture. Scale bars, 25 μm.

incubation at 37 °C for 1 h. Next, fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories) was applied at a 1:35 dilution with incubation at 37 °C for 1 h. Coverslips were mounted in glycerol at *p*H 8.5, and viewed and photographed under a Zeiss fluorescence and phase-contrast microscope. Specificity was demonstrated by absence of specific staining when the rabbit anti-somatostatin at 1:100 dilution was adsorbed with an equal volume of 10^{-3} M somatostatin before use. Staining was unaffected by adsorption with gonadotropin releasing hormone, vasopressin or ACTH.

Dichter has previously reported that by using a variety of confirmatory techniques (including silver staining and intracellular recordings), neurones can be recognised in the cultures by their appearance under phase-contrast[6]. All the cells shown by the indirect immunofluorescent technique to contain IR-somatostatin in these cultures were morphologically indistinguishable from neurones identified by these other criteria.

An estimated 3–5% of the neuronal population stained unequivocally in each culture. Most somatostatin-positive cells had two or more large dendritic processes, but by phase-contrast microscopy these positively staining cells could not be distinguished from non-somatostatin-containing neurones. Within cell bodies, IR-somatostatin was predominantly located in a perinuclear distribution which often seemed continuous with staining in the proximal portions of cell processes (see Fig. 2). In axonal processes the staining was most frequently seen in varicosites, and a densely stained collection was often seen at the most distal portion of the process.

These results suggest that neurones of the mammalian cerebral hemispheres can produce somatostatin completely independently of the hypothalamus. The finding of a larger form of IR-somatostatin corresponds with similar findings in many other nervous and gastrointestinal tissue homogenates, including extra-hypothalamic brain[3,7]. The presence of this larger form in the cellular fraction, and not in the media, suggests a prohormone which is not secreted as such, but pulse-chase studies must be made before the presence of such a precursor can be confirmed.

Many studies have reported neurophysiological effects of somatostatin; it depresses the firing of neurones in hypothalamus, cerebellar cortex and cerebral cortex[8], and in brainstem, reticular formation and dorsal hippocampus[9]. By contrast, somatostatin has been shown to excite cortical neurones in conscious rabbits[10] and in a rat hippocampal slice preparation[11]. Its release by calcium-dependent depolarisation has been demonstrated in several neural structures, including hypothalamus[12,13], neurohypophysis[14] and dorsal root ganglia[15]. Our findings support the view that somatostatin may have a role as an extra-hypothalamic central nervous system neurotransmitter or neuromodulator.

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Cholesterol levels inversely reflect the thermal sensitivity of mammalian cells in culture

Anne E. Cress & Eugene W. Gerner

The University of Arizona Health Science Center, Departments of Biochemistry and Radiology/Radiation Oncology Division, Tucson, Arizona 85724

Cholesterol is a primary component of the mammalian cell plasma membrane. Although its function is unknown, it may be of major importance in maintaining membrane fluidity and rigidity[1]. In artificial membrane systems, the addition of cholesterol results in a condensing effect—thickening the bilayer and inducing higher order in the acyl chains of the phospholipids[2]. Permeability profiles indicate that the addition of cholesterol into egg-lecithin bilayers increases the half-time of solute transport[3]. In addition, decreased amounts of sterol in the membrane increase glucose permeability, and, in L cells, increase the transport of rubidium[4,5]. These studies suggest a role for cholesterol in changing the physical characteristics of the membrane resulting in the alteration of membrane permeability. We now provide evidence that cholesterol may act, presumably via changes in physical membrane properties, with yet another biological consequence; regulating the survival sensitivity of mammalian cells to hyperthermic temperatures.

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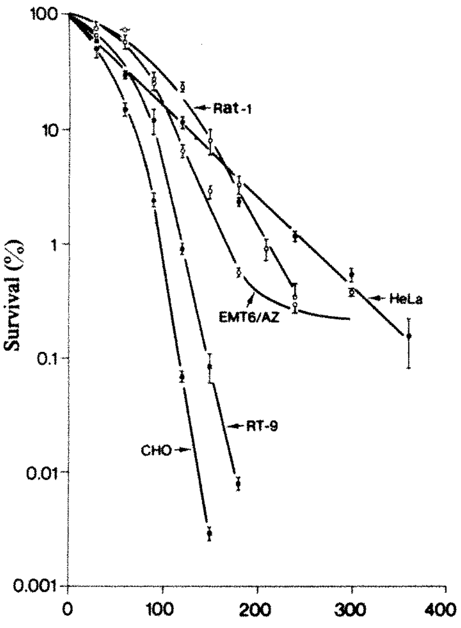

Fig. 1 Thermal survival response of exponentially growing cells in culture. (●), HeLa; (○), EMT6/AZ; (■), RT-9; (□), Rat-1; (×), CHO. After exposure of the cells to 43 °C for the indicated times, cells were collected and plated for colony formation. Standard errors of survival determinations are shown.

Elevated temperatures are known to result in the modification of physical membrane properties in prokaryotic cells, principally by alteration of the amounts of saturated and unsaturated fatty acids[6]. Yatvin[7] has shown that dietary manipulation of the proportion of saturated and unsaturated fatty acids in *Escherichia coli* membranes acts to modify the thermal sensitivity of these organisms. Prokaryotic membranes contain essentially no sterol and the membrane composition in general is quite different from eukaryotes[8]. However, the physical changes of the prokaryotic membrane during the acclimation to elevated temperatures are remarkably similar to the changes in the mammalian cell membrane when cholesterol is introduced. In both cases, the membranes become more ordered and less permeable.

We have previously shown that one immediate response to exposure to elevated temperatures is an increase in plasma membrane permeability[9]. Recently, we have demonstrated that modification of membrane permeability can also lead to modification of cellular viability at hyperthermic temperatures[10].

Guided by these observations, we added whether the known different heat sensitivites of various mammalian cell lines in culture[11] would reflect differences in the amount of cholesterol in the unheated cells. One would expect that elevated levels of cholesterol in untreated cells might be expressed as a higher survival rate following hyperthermia treatments. Hyperthermia in this study represents an elevation of temperature from 37 °C to 43 °C.

Cultures of HeLa, EMT6/AZ, Rat-1, RT-9, and CHO cell lines (see Table 1 for identification of cells lines) were maintained and treated in log phase growth in McCoy's 5A medium supplemented with 20% fetal calf serum, 100 units ml^{-1} penicillin and 100 μg ml^{-1} streptomycin (all from GIBCO). All lines were grown in a 5% CO$_2$–95% air atmosphere at 37 °C. Doubling times, under these conditions for the HeLa, EMT6/AZ, Rat-1, RT-9, and CHO cells, were 20–22, 15–16, 15–17, 14–15, and 13–15 h, respectively. Colony-forming efficiencies for untreated cells growing at 37 °C, for these same lines were 60–80%, 60–80%, 50–70%, 60–80%, and 80–95% respectively.

Cell survival, determined by the colony-forming ability of single cells, was used as the index of the thermal sensitivity of the various cell lines studied. The procedure we used, and the hyperthermic treatment conditions in our temperature-controlled water bath (temperature uniform and constant to ±0.1 °C), have previously been described in detail[12,13].

For cholesterol determination, 2.5×10^7 cells per sample were collected by scraping with a 'rubber policeman', washed in Puck's saline A (GIBCO), and sonicated into 0.01 M Tris buffer, *p*H 7.2 and centrifuged for 20 min. at 20,000 r.p.m. The particulate fraction was extracted with chloroform : methanol : water (2 : 2 : 1) and the amount of cholesterol determined spectrophotometrically by the method of Glick *et al.*[14]. Cholesterol (Sigma) was used as the standard and the extraction procedure was checked by thin layer chromatography using a chloroform : methanol : acetic acid (90 : 10 : 1) solvent. Protein determinations were done according to the Bradford method[15]. Phospholipid concentrations were measured from the extract of the particulate fraction by the colorimetric method of Raheja *et al.*[16]. A chromatographically pure reference standard of phosphatidyl choline (Sigma) was used.

The molar ratios of saturated to unsaturated fatty acid represents 18 : 0/18 : 1. Approximately 1.0×10^6 cells were used for this particular assay. After the particulate fraction was isolated (as previously described) 1 ml of 2.5 M KOH in methanol and 40 μg of heptadecanoic acid (an internal standard) were added and this mixture was heated for 30 min at 70 °C. After cooling, 1.5 ml of 1 M phosphoric acid was added and mixed well. Then 1 ml of hexane was added to the hydrolysate, mixed well, and centrifuged at 3,000 r.p.m. for 3 min. Two additional 0.5 ml hexane extractions were carried out and all the hexane solutions were combined. To the hexane solution, 200 μl of 0.5 M aqueous trimethyltrifluorotolylammonium hydroxide[17] were added. Fatty acid methylesters were formed on the gas chromatograpic column (2 mm × 0.5 M, 3% Silar 10C on Gas Chrom Q 100/120) which was interfaced with a mass spectrometer (Finnigan 3300). The fatty acid methylesters were quantified by their relative weight response to the internal standard using the total ion current of the selected ions.

Figure 1 shows the per cent survival of each cell line as a function of time at 43 °C. Note that all cell lines were grown in identical medium since it is known that exogenous cholesterol affects the cellular biosynthesis of the sterol[18]. Under these conditions, cellular sensitivity to a heat challenge is different for each cell line studied. HeLa cells appear to be the most thermal resistant cell line and CHO the most thermal sensitive of the lines tested. Also, most lines display a non-exponential survival response ('shoulder region') which precedes the exponential decrease in cell survival as time at 43 °C increases. The HeLa cells are an exception to this case, while the EMT6/AZ cells deviate from strictly exponential cell killing at longer treatment times. The plateau in the survival response for EMT6/AZ cells, after 200 min of hyperthermic exposure, represents the development of heat resistance and has been studied in detail for this line[19].

Comparisons of the differences in thermal sensitivity between these lines can be made by determining the D_0, a common

Table 1 Cholesterol content in the particulate fraction of untreated cells growing at 37 °C and the sensitivity of these cells to 43 °C

Cell line	Source	Cholesterol (μg per mg protein)	D_0 (43 °C) (min)
HeLa	Human cervix carcinoma	54.06 ± 1.50	54
Rat-1	Rat embryo fibroblast	33.90 ± 0.15	28
EMT6/AZ	Mouse mammary sarcoma	33.74 ± 0.98	24
RT-9	Rat astrocytoma	20.66 ± 2.86	12
CHO	Chinese hamster ovary	18.63 ± 0.41	10

parameter in the radiobiological literature, for each survival response. The D_0 value is simply the reciprocal slope of the exponential portion of the survival curve, and in this case, has units of time for a specific temperature. Thus, a survival curve with a higher D_0 than that of another indicates relative resistance to the particular treatment.

Table 1 lists the sources of the cell lines tested, the respective D_0 value for each cell line, which was calculated from Fig. 1, and the amount of cholesterol found in untreated cells. The quantity of cholesterol is expressed in terms of protein amounts found in the cell particulate fraction. There appears to be a remarkable correlation between D_0 and the cholesterol content. This correlation is still valid when the cholesterol content is related to cell number (data not shown). Since D_0 is inversely related to the survival sensitivity of treated cells, these data show that cholesterol content is inversely correlated with hyperthermic sensitivity. In addition, we investigated whether D_0 would also correlate with the cholesterol-to-phospholipid molar ratio or the degree of saturated to unsaturated fatty acid found in the particulate fractions of the cell lines under study. This is important since these various membrane parameters are also known to affect the physical state of the membrane and might just as easily correlate with the respective D_0 value. Table 2 gives the cholesterol-to-phospholipid molar ratio and the molar ratio of 18:0 to 18:1 observed in four of the nine cell lines tested. It is important to note that the ratio of saturated to unsaturated fatty acids does not follow any significant pattern with the amount of cholesterol present in each cell line. Our interpretation is that the best correlate with the survival response to hyperthermia (D_0) is still the amount of cholesterol in the particulate fraction.

Table 2 Relationship between heat sensitivity at 43 °C, cholesterol : phospholipid molar ratio, and the ratio of saturated to unsaturated fatty acid (18 : 0/18 : 1)

Cell line	Heat sensitivity at 43 °C (D_0) (min)	Cholesterol : phospholipid molar ratio	Saturated: unsaturated fatty acid molar ratio
Rat-1	28	0.264	1.4
EMT6/AZ	24	0.657	0.86
RT-9	12	0.470	1.0
CHO	10	0.502	1.4

As described in Fig. 2, this correlation is expressed as a nearly linear relationship between the respective D_0 value for each cell line and the measurable cholesterol present in the cell particulate fraction. This linear relationship is impressive, since the thermal survival sensitivity does not correlate with any other obvious parameters of the lines tested, such as species, doubling time or colony-forming abilities, as mentioned earlier, of the untreated cells. Nor does survival sensitivity correlate directly with the tumorogenicity of the cell lines tested, since the Rat-1 and CHO cell lines are of normal tissue origins, while the HeLa, RT-9, and EMT6/AZ cell lines are of tumour origins. The RT-9 and EMT6/AZ cell lines are normally maintained by sequential *in vivo*/*in vitro* passage and form solid tumours when grown in F344 rats and BALB/c mice, respectively. It should be noted here that Green estimates that 95% of the cellular cholesterol is located in the plasma membrane[20]. Therefore, it is reasonable to presume that the cholesterol content measured here represents plasma membrane cholesterol.

The demonstration of increased cholesterol content in cells that are relatively resistant to elevated temperatures (HeLa compared to CHO) is interesting because it suggests that the sterol provides a similar function to the saturation of fatty acids in prokaryotes. In addition, since hyperthermia is presently used as an anticancer agent, this finding offers the possibility of a predictive tool for determining individual tumour susceptibility to subsequent hyperthermia therapy.

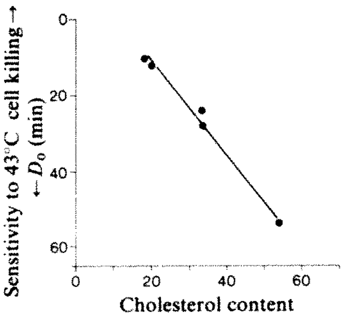

Fig. 2 Relationship between cholesterol content in untreated cells and 43 °C survival sensitivity. The values for the respective D_0 and cholesterol content of each cell line were taken from Table 1. This emphasises in graphical form the apparent linear relationship between cellular sensitivity to 43 °C and the cholesterol content in untreated cells.

In either case, it is important to test further the correlation of cholesterol, and other factors affecting membrane structure and permeability, and heat resistance. In this regard, it is of interest that the cholesterol : phospholipid molar ratios and the saturated : unsaturated fatty acid molar ratios do not correlate with various heat sensitivities of the cell lines tested. Studies to be published elsewhere do, however, show that the saturated : unsaturated fatty acid molar ratio does correlate with cell survival sensitivity at 43 °C, within one cell line, when the normal growth temperature is either increased or decreased from 37 °C. These studies emphasise that a number of factors affecting membrane structure and function may be involved in cellular responses to hyperthermia and that the normal growth temperature may be important in determining which of these factors are limiting. We are also extending the studies in this report by asking if cholesterol content is also elevated under conditions of induced thermal resistance[12]. Preliminary experiments demonstrate that this is the case[10], which in turn suggests that the activity of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis, should also be increased in thermal-resistant cells.

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The position of tropomyosin in muscle thin filaments

J. Seymour & E. J. O'Brien

Medical Research Council Cell Biophysics Unit, Department of
Biophysics, King's College, 26–29 Drury Lane, London WC2, UK

The contraction of vertebrate skeletal muscle is controlled by the action of Ca^{2+} on muscle thin filaments. At low Ca^{2+} concentrations ($<10^{-6}$ M) the regulatory proteins of the thin filament, tropomyosin and troponin, relax muscle by preventing the interaction of myosin and actin. At higher Ca^{2+} levels this inhibition is removed, when Ca^{2+} binds to troponin. Tropomyosin, a long coiled-coil α-helical molecule, is located in each of the two long-pitch helical grooves of actin, but troponin, a globular molecule, is attached at intervals of 38 nm (refs 1, 2). By combining evidence from X-ray diffraction studies of muscle and from electron microscopy, several authors have proposed that tropomyosin moves to block or allow attachment of myosin heads to actin[3–5]. We investigate here an alternative way of combining the data, which if valid may have important consequences for our understanding of the regulation of muscle contraction[6].

Figure 1 illustrates the problem. The shape and attachment site of myosin subfragment-1 (S-1) are based on a three-dimensional reconstruction of electron micrographs of the S-1–thin filament complex calculated by Moore *et al.*[7]. This reconstruction does not have sufficient resolution to reveal tropomyosin, but its position may be derived from analysis of X-ray diffraction patterns of muscle[3–5], or image analysis of electron micrographs of reconstituted thin filaments[8,9]. However, as pointed out by previous authors[3,8], these studies have not shown on which side of the actin groove tropomyosin is located relative to the S-1 attachment site. To remove this ambiguity, it is necessary to superimpose, with the correct relative orientation, reconstructions of decorated and undecorated thin filaments, the latter showing tropomyosin well resolved. If reconstructions are carried out on micrographs of I-segments, the assemblies of thin filaments still attached to the Z-line, the correct orientation is known, because the characteristic arrowhead appearance obtained by adding S-1 to thin filaments always points away from the Z-line[10].

An I-segment from a crude muscle homogenate obtained at a low Ca^{2+} concentration is shown in Fig. 2*a*. Some homogenates at a high Ca^{2+} level were studied, but acto-myosin precipitation made these experiments difficult. At low Ca^{2+} concentrations, all preparations produced up to 80% inhibition of the acto-myosin ATPase rate, confirming the presence of regulatory proteins. Furthermore, troponin gave rise to strong meridional reflections at 1/38.5 nm^{-1} in the Fourier transforms of many micrographs and also gave rise to corresponding periodic stripes visible in large I-segments. Side-by-side aggregation of filaments into paracrystals was induced by raising the Mg^{2+} concentration, but single-layered paracrystals of more than two or three filaments were rare because movement of the filaments was restricted by their Z-line attachment and tangling readily occurred. Filaments within paracrystals are usually better for image analysis studies because they are straighter, more evenly stained and less flattened[11].

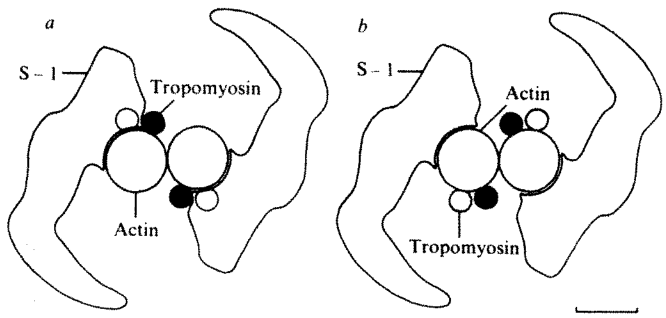

Fig. 1 The two ways of superimposing reconstructions from decorated (that is, complexed with S-1) and undecorated thin filaments. *a*, Based on Huxley[3]. *b*, As *a* except that the thin filament has the opposite orientation. The arrowheads are pointing out of the plane of the paper away from the Z-line. Tropomyosin in contracting muscle is represented by filled circles and in relaxed muscle by open circles. Scale bar, 5 nm.

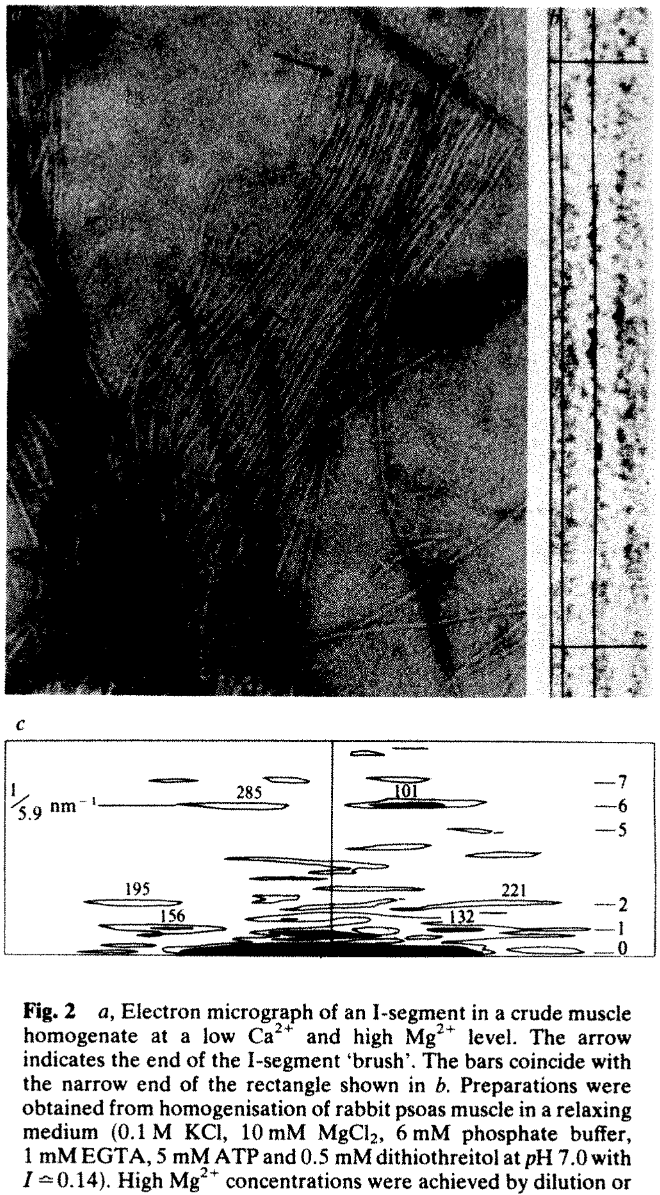

Fig. 2 *a*, Electron micrograph of an I-segment in a crude muscle homogenate at a low Ca^{2+} and high Mg^{2+} level. The arrow indicates the end of the I-segment 'brush'. The bars coincide with the narrow end of the rectangle shown in *b*. Preparations were obtained from homogenisation of rabbit psoas muscle in a relaxing medium (0.1 M KCl, 10 mM $MgCl_2$, 6 mM phosphate buffer, 1 mM EGTA, 5 mM ATP and 0.5 mM dithiothreitol at *p*H 7.0 with $I \simeq 0.14$). High Mg^{2+} concentrations were achieved by dilution or dialysis into the above relaxing medium, except KCl was replaced by $MgCl_2$ to give an overall $I \simeq 0.15$. Some preparations were diluted or dialysed into a high Ca^{2+} concentration medium (0.1 M KCl, 6 mM $MgCl_2$, 4 mM ATP, 2 mM $CaCl_2$ and 10 mM ImCl at *p*H 7.3 and $I \simeq 0.13$). Specimens were mounted on carbon-coated copper grids and negatively stained with 1% uranyl acetate. Magnification ×88,000. *b*, Display of densitometered region of *a*. The marked rectangle encloses the area Fourier transformed in *c*. *c*, Fourier transform of single filament shown in *b*. Amplitude peaks are contoured and numbers above layer-line peaks indicate phases. Layer-line numbers shown on right hand side are those arising from an actin filament with 13 subunits in 6 turns of the genetic helix[7].

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Only filaments that seemed to be connected to a Z-line and terminated at the end of the I-segment 'brush' were considered for analysis. Optical diffraction patterns of electron micrographs of many hundreds of such filaments were examined, but only 20 patterns clearly showed at least three actin layer lines. The density distribution of these micrographs was converted into digital form and their Fourier transforms calculated[11] (Fig. 2*b*, *c*). The quality of the phase and amplitude information justified three-dimensional reconstruction in eight cases, where for example, the phase residual was always less than 37° and had an average value of 28°. The phase residual (see Table 1) is a measure of the amount by which the helical symmetry deviates from that expected for actin[7], and was calculated for all non-equatorial layer-line data of medium or strong intensity. A high phase residual was associated with asymmetries between the upper and lower sides of the filaments and the relatively poor signal:noise ratio, arising from additional proteins in the homogenate and filament curvature restricting the length of filaments processed. Straight filaments not in paracrystals sometimes gave low phase residuals, but in most cases it was better to mask off a filament within a paracrystal or to deconvolute the Fourier transform of the paracrystal to give the transform of an average filament. Six reconstructions clearly resolved the relative positions of actin and tropomyosin, in one case the Ca^{2+} level was high. By orientating each reconstruction with respect to the Z-line, tropomyosin was consistently found to occupy positions shown in Fig. 1*b*, that is, on the opposite side of the actin groove to that of the postulated S-1 binding site.

Two reconstructions can be calculated from any set of Fourier transform data, using layer-line information arising from either the upper or lower sides of the filament[12]. In Table 1 the discrepancies between the peak amplitudes measured on the left and right hand sides of the meridian and the sometimes large value of $(\Delta\theta \mp \pi n)$ indicate a difference in the stain distribution on the two sides of the filament. The data corresponding to each side were therefore used independently to calculate three-dimensional reconstructions, and in each case the assignment of the actin and tropomyosin positions was the same. Additionally, model calculations indicated that phase changes on the first and second layer lines of two to three times the average phase residual would be required to move the tropomyosin from one side of the actin groove to the other.

Table 1 Peak amplitude and phase data

Filament reference no.	Actin layer-line no.[7]	Peak amplitudes (arbitrary scale, not normalised)		$\Delta\theta$ $\mp\pi n$	Phase residual
		LHS	RHS		
	1	115	217	36°	
1	2	108	52	32°	28°
	6	172	165	9°	
	1	164	245	4°	
2	2	76	151	28°	20°
	6	172	137	1°	
	1	143	162	4°	
3	2	85	125	88°	25°
	6	117	141	8°	
	1	129	201	14°	
4	2	83	94	11°	37°
	6	141	147	27°	
	1	187	167	18°	
5	2	119	127	29°	24°
	6	203	118	11°	
	1	98	96	56°	
6	2	75	79	1°	31°
	6	73	124	46°	

The data for filament 2 were obtained from the deconvoluted Fourier transform of a paracrystal. In other cases, the data shown are from the transform of masked-off filaments. For filaments 4 and 5, the deconvolution analysis was also carried out and gave results not significantly different from those shown. $\Delta\theta$ Is the phase difference between Fourier coefficients along a layer line on opposite sides of the meridian and at a radial spacing equal to the average radial spacing of the peak amplitude on each side of the meridian. n Is the principal Bessel order expected on the layer line in question. The phase residual is normalised, amplitude weighted $(\Delta\theta \pm \pi n)$ using all Fourier coefficients whose amplitudes are significantly above the background noise level, as described by Wakabayashi *et al.*[8] (where it is termed a 'self residual' Q). The full amplitude contour map for filament 5 is shown in Fig. 2*c*.

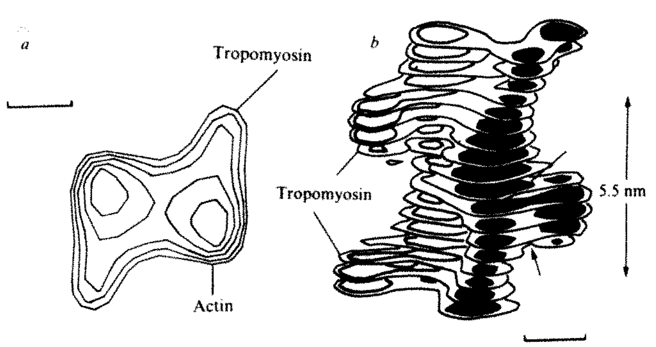

Fig. 3 *a*, Contour map of the helical projection derived from averaged layer-line data. The helical projection is formed by projecting the structure down the long-pitch (72 nm) actin helices looking towards the Z-line. *b*, Three-dimensional reconstruction from averaged layer-line data, with the Z-line at the bottom. The different shadings indicate the long-pitch actin and tropomyosin strands on opposite sides of the helical axis. Actin layer lines[7] used in these reconstructions included $l = 0, 1, 2, 3, 5, 6, 7$ and 8. The equator was used at full weight and meridional reflections on the first layer line were weighted down[8]. Data were derived from three transforms originating from filaments at low Ca^{2+} concentrations. Scale bars, 2 nm.

Five sets of data from three filaments (2, 3 and 5 of Table 1) were averaged by the methods of Amos and Klug[13], and Fig. 3*a* shows the resulting helical projection. The averaging produced an improvement in the signal:noise ratio of the data, particularly on weaker layer lines. Great care was taken to preserve the known orientation of each filament and the effect of each mathematical operation was monitored on a test object, which was an S-1-decorated thin filament having an obvious polarity. The helical projection shows on each side a well resolved actin peak with, extending to higher radius, a smaller region of lower density attributable to tropomyosin. This assignment is confirmed by the three-dimensional analysis (Fig. 3*b*), where the size and shape of the actin subunit are consistent with that observed in other reconstructions[8]. In common with earlier reconstructions[14], tropomyosin seems to be discontinuous, which may be due to inadequate resolution and the periodic merging of tropomyosin with part of the actin subunit. Where tropomyosin diverges from one end of the actin subunit a V-shaped cleft is formed (arrow, Fig. 3*b*). Furthermore, each actin subunit makes a connection to each of the two adjacent subunits in the other long-pitch strand and these are asymmetrically disposed with respect to the subunit's centre of mass. These features are present in the reconstructions of Wakabayashi *et al.*[8] and assist in assigning filament polarity.

These reconstructions remove an important ambiguity present in all previous structural work as to which side of the actin groove tropomyosin occupies. It is not known whether the azimuthal movement of tropomyosin regulates contraction by sterically blocking incoming myosin heads, or by changing the conformation of actin subunits so that they cannot bind myosin. The steric blocking model was formulated on the assumption that tropomyosin occupies positions shown in Fig. 1*a*, which we have found to be erroneous. In Fig. 1*b* there is no obvious way in which tropomyosin can sterically block myosin, unless the shape of S-1 or its position of attachment to actin is revised. We understand that K. Taylor and L. A. Amos (personal communication) are now re-examining the structure of decorated thin filaments and their results will be of particular interest.

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Electron transfer reagent binding sites on plastocyanin

David J. Cookson, Mark T. Hayes & Peter E. Wright

Department of Inorganic Chemistry, University of Sydney, Sydney, NSW 2006, Australia

Electron transfer is an essential process in all biological systems. The kinetics of electron transfer reactions between small inorganic complexes and a variety of metalloproteins have been extensively studied[1–5]. Recent studies on the electron transfer reactions of plastocyanin, an essential component of the photosynthetic electron transport chain, have shown[6–9] that inorganic redox reagents such as the hexacyanoferrate(III) ion ($Fe(CN)_6^{3-}$) and the tris(1,10-phenanthroline)cobalt(III) ion ($Co(1,10\text{-phen})_3^{3+}$) form discrete complexes with the protein before the electron transfer step. Here, we have used high resolution nuclear magnetic resonance spectroscopy (NMR) to study the interaction of plastocyanins from French bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*) with the chromium(III) analogues of these redox reagents, $K_3Cr(CN)_6$ and $Cr(1,10\text{-phen})_3(ClO_4)_3$. The binding of these complexes to plastocyanin is highly specific and we identify two binding sites, separated by about 15 Å, which are apparently determined primarily by electrostatic interactions. Two distinct electron transfer pathways to and from the copper atom are indicated.

Plastocyanin is a type 1 or 'blue' copper protein of molecular weight 10,500. It contains one copper atom, in a distorted tetrahedral environment, which is coordinated by the thiol group of Cys 84, the thioether group of Met 92 and the imidazole groups of His 37 and His 87 (ref. 10). Its low molecular weight makes it ideally suited to study by high resolution NMR spectroscopy and to date the resonances of many amino acids near the copper atom have been identified[11–14]. These resonances, which are readily detected in the spectrum of the copper(I) protein, are broadened beyond detection when the protein is in the copper(II) state. The aromatic region of the proton NMR spectrum of reduced French bean plastocyanin is shown in Fig. 1*a*. The resonances of both histidine residues, all three tyrosine and two phenylalanine residues have been identified previously[13]. With the help of the X-ray crystal structure[10], assignments to specific amino acids can now be made. Full details will be published separately but we note here that coupled doublets at 6.51 and 6.64 p.p.m., which arise from the tyrosine residue closest to the copper atom[13], can, from the X-ray structure[10], be assigned to Tyr 83. Specific assignments of the histidine resonances have also been made. On the basis of their pronounced *p*H dependence (ref. 11 and our own unpublished observations), resonances at 7.66 and 6.97 p.p.m. are assigned to the C-2 and C-4 protons[13], respectively, of His 87 which is exposed to the solvent at one end of the molecule[10]. The other, more deeply buried histidine (His 37) must thus give rise to the resonances[13] at 7.57 and 7.16 p.p.m. which show only slight dependence on *p*H. The spectrum of the French bean protein has many features in common with those of other plastocyanins and the protein structure, particularly in the vicinity of the copper atom, is expected to be conserved in plastocyanins from all higher plant species[13].

The effect of addition of $Cr(CN)_6^{3-}$ to a solution of reduced French bean plastocyanin is shown in Fig. 1*b*. Significant changes in the NMR spectrum occur and are best seen in the difference spectrum shown in Fig. 1*c*. At low concentrations of $Cr(CN)_6^{3-}$, the resonances of His 87 broaden. As the concentration of $Cr(CN)_6^{3-}$ is increased, the resonances of His 37 also begin to broaden. In the aliphatic region of the spectrum (not shown), the resonance of Met 92 (a copper ligand) at 0.57 p.p.m. is broadened slightly.

Perturbations are also observed in the NMR spectrum of reduced plastocyanin when the protein is titrated with $Cr(1,10\text{-phen})_3^{3+}$ (Fig. 2). However, these perturbations differ from those observed with $Cr(CN)_6^{3-}$. The resonances of Tyr 83 are broadened dramatically, the effect being most noticeable at a $Cr(1,10\text{-phen})_3^{3+}$ concentration of 0.4 mM (3.5 mM plastocyanin). In the aliphatic region of the spectrum, the methyl group proton resonances of two or three (as yet unidentified) amino acid residues are broadened. These resonances are not broadened by $Cr(CN)_6^{3-}$.

The different perturbations of the NMR spectrum induced by $Cr(CN)_6^{3-}$ and $Cr(1,10\text{-phen})_3^{3+}$ indicate that these reagents bind to plastocyanin at two different sites. The broadening of resonances results from an enhancement of nuclear relaxation brought about by dipolar coupling with the relatively slowly relaxing electron spin of the Cr(III) ion. The broadening is dependent on the inverse 6th power of the distance of the amino

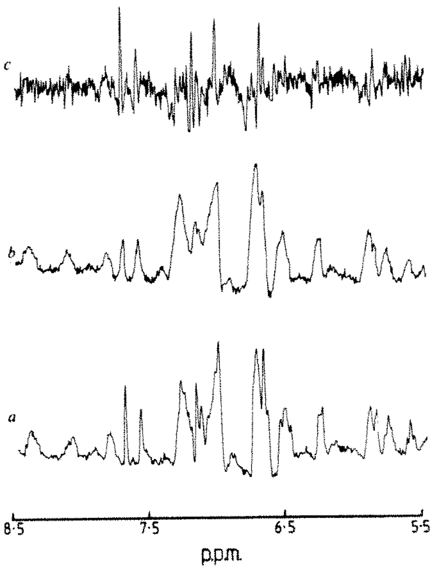

Fig. 1 Aromatic region of the resolution-enhanced NMR spectrum of French bean plastocyanin (3.5 mM, *p*H 6.3, 50 mM phosphate buffer in D_2O) in the presence of no $Cr(CN)_6^{3-}$ (*a*) and 0.4 mM $Cr(CN)_6^{3-}$ (*b*). *c*, Difference spectrum (*a* − *b*). In *a* and *b* the resolution has been enhanced by means of the convolution difference technique[16]. The residual HOD peak was suppressed by selective gated irradiation. Dioxan was used as an internal standard but all peaks are referred to TSS (trimethylsilylpropanesulphonic acid). The *p*H values quoted are uncorrected meter readings. Plastocyanins were prepared by a modification of the method of Ramshaw *et al.*[17].

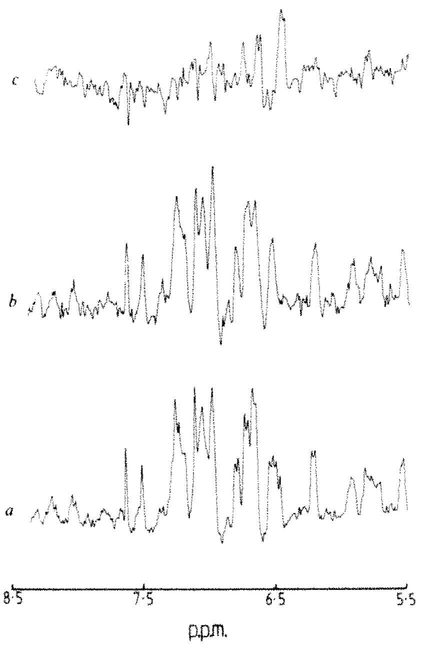

Fig. 2 Aromatic region of the resolution-enhanced NMR spectrum of cucumber plastocyanin (3.5 mM, *p*H 7.0, 50 mM phosphate buffer in D_2O) in the presence of no Cr(1,10-phen)$_3^{3+}$ (*a*) and 0.4 mM Cr(1,10-phen)$_3^{3+}$ (*b*). *c*, Difference of direct spectra obtained before and after addition of 0.4 mM Cr(1,10-phen)$_3^{3+}$.

acid residue(s) from the paramagnetic centre. Consequently, any resonances broadened by the addition of the Cr(III) complexes must arise from amino acid residues close to the sites of binding to the protein. One site, to which Cr(CN)$_6^{3-}$ binds, must lie close to the copper atom itself, as the resonances of three of the copper ligands are broadened. The resonances of His 87 are observed to broaden first. The copper atom lies at one end of the protein molecule in a depression lined by conserved hydrophobic residues and is protected from solvent by the imidazole group of this histidine residue[10]. It is conceivable that positive charge from the copper atom is delocalised over this imidazole ring which would be sufficient to bring about an electrostatic interaction with Cr(CN)$_6^{3-}$.

The second site must lie in the vicinity of Tyr 83 because the resonances of this residue are broadened by the binding of Cr(1,10-phen)$_3^{3+}$. The side chain of Tyr 83 lies close to the surface of the protein about 10 Å from the copper atom and is adjacent to a cluster of carboxylate groups[10] belonging to conserved acidic residues. This 'pocket' of negative charge is unique in plastocyanin and clearly provides a favourable binding site for positively charged reagents. Our NMR studies show that Cr(NH$_3$)$_6^{3+}$ also binds at this site (D.J.C., M.T.H. and P.E.W., unpublished observations).

Our results suggest that the charge on the inorganic reagent has a dominant role in determining the site of interaction on the protein. Sykes and coworkers[9] have shown that oxidation reactions involving Co(1,10-phen)$_3^{3+}$ are blocked by Cr(1,10-phen)$_3^{3+}$, indicating conclusively that these reagents compete for a single binding site on the protein from which electron transfer occurs. Furthermore, we expect Cr(CN)$_6^{3-}$ to bind at the same site as Fe(CN)$_6^{3-}$ in view of their similar size and identical charge and ligands. The existence of two binding sites for redox reagents on plastocyanin is not unexpected in view of the electron transport function of the protein[15].

The rate constants for the reactions of parsley plastocyanin with Fe(CN)$_6^{3-}$ and Co(1,10-phen)$_3^{3+}$ are both *p*H dependent but have different pK_a values (5.7 and 6.1, respectively)[7,9]. In addition, the reciprocal of the first order rate constant, k_{obs}, is inversely proportional to the oxidant concentration[7]. These observations led Sykes and coworkers to conclude that productive complexes are formed before electron transfer and that at least two binding sites are involved which they supposed to be in the same locality of the protein[9]. In our present NMR studies we have identified these two binding sites on plastocyanin. One is close to the copper atom and the other is at least 10–15 Å away. This observation confirms earlier suggestions based on the X-ray crystal structure[10] that two electron transfer pathways to and from the copper atom are used in the redox reactions of plastocyanin and that possibly two mechanisms of electron transfer are involved.

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The β-globin gene is on the short arm of human chromosome 11

Linda Sanders-Haigh, W. French Anderson & Uta Francke*

Laboratory of Molecular Hematology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205
*Department of Human Genetics, Yale University School of Medicine, New Haven, Connecticut

Investigations on the regulation of human globin gene expression are assisted by a knowledge of the chromosomal location of the globin genes. Previous studies[1] have mapped the α-globin locus to human chromosome (HC) 16, and have shown that the human globin gene complex γ-δ-β co-segregates with lactate dehydrogenase A (LDH-A) and the presence of HC 11 in somatic cell hybrids[2,3]. Radioactively labelled globin complementary DNA (cDNA) probes were used in molecular hybridisation experiments to determine the chromosomal locations of the α- and β-globin genes. When human × rodent somatic cell hybrids are used which contain well-defined parts of human chromosomes, direct mapping of genes of chromosomal regions or single bands is possible[4,5]. We have regionally localised the human β-globin gene using two sets of hybrid cell lines: (1) Chinese hamster × human hybrid cells containing the HC 11 long arm or both the short and long arms and (2) mouse × human hybrids containing only the HC 11 short arm. The techniques of liquid molecular hybridisation[3] and Southern blotting[6] with ^{32}P-labelled human β–globin cDNA (from plasmid JW102)[7] have been used to localise the β-globin gene sequence to region 11p11 → 11p15. Similar results were reported recently by Jeffreys *et al.*[8].

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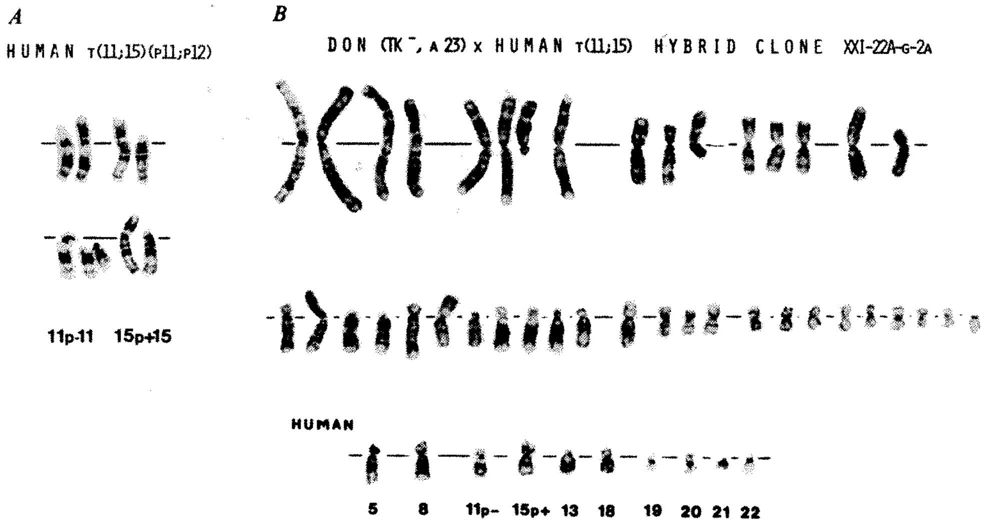

Fig. 1 *A*, Chromosome pairs 11 and 15 showing balanced reciprocal translocation t(11; 15) (p11; 12). The rearranged chromosomes der(11), or 11p-, and der(15), or 15p+, are placed to the left and the normal homologues 11 and 15 to the right of each pair. Trypsin–Giemsa banding was carried out according to Francke and Oliver[12]. *B*, Karyotype of Chinese hamster (Don, TK⁻) × human t(11; 15) hybrid clone with trypsin–Giemsa banding. The human chromosomes are arranged in the bottom line. Both translocation chromosomes are present but the normal chromosome 11 has been lost.

Chinese hamster × human cell hybrids containing the long arm of HC 11 were obtained when skin fibroblasts from a balanced carrier of a 46, XY, t(11; 15) (p11; p12) translocation (Fig. 1*A*) were fused to Chinese hamster Don cells (a23; TK⁻) using polyethylene glycol as a fusogen[9]. Cell hybrids were selected in hypoxanthine/aminopterine/thymidine (HAT) medium. The translocation chromosomes der(11) or 11p- (region 11p11 → qter) and der(15) or 15p+ (region 11pter → p11) and the normal HC 11 segregated randomly. After screening many primary hybrid clones for their content of chromosomes containing HC 11 material, and after two rounds of subcloning, we have isolated a number of independent primary and secondary hybrid cell lines which have retained the der(11) chromosome at high frequency and have lost the normal HC 11 and the der(15) translocation product. These clones do not express human LDH-A, while others containing both translocation chromosomes after loss of the normal HC 11 (Fig. 1*B*), are positive for LDH-A expression. These results are consistent with the previous localisation of LDH-A to band 11p12 (ref. 10).

Mouse × human somatic cell hybrids containing the short arm of HC 11 were obtained when BALB 3T3 cells deficient in thymidine kinase (TK) were fused to human fibroblasts with a t(11; 17) (p15; q21) balanced translocation. In subclones a secondary rearrangement was identified and characterised as 11cen → 11p15 :: 17q21 → 17qtr (mar-1). This telocentric marker chromosome carries the human gene for TK (in region 17q21 → 17qtr) and the gene for LDH-A (in region 11cen → 11p15)[4]. This chromosome is selectively retained in cell hybrids grown in HAT medium because it carries the human TK gene, complementing the enzyme defect in the murine parental cell line. In fact, mar-1 was the only human chromosome retained in hybrid clones VII-2,3 and 4 HAT, as demonstrated by Hoechst 33258 and Giemsa 11 staining. (A cell line derived from one of these hybrid clones has been designated LBP-2 in the laboratory of Dr Walter Bodmer and has been used by Jeffreys *et al.* in similar experiments to those described here[8].) After counter selection in 5-bromodeoxyuridine (BUdR)-containing medium, derivative clones lacking mar-1 have been obtained (VII-2 and 4, BUdR)[11]. These back-selected clones no longer express LDH-A.

Hybrid clones from both sets of experiments were characterised cytologically by trypsin–Giemsa banding and karyotyping (Fig. 1*A*, *B*), and biochemically by LDH electrophoresis

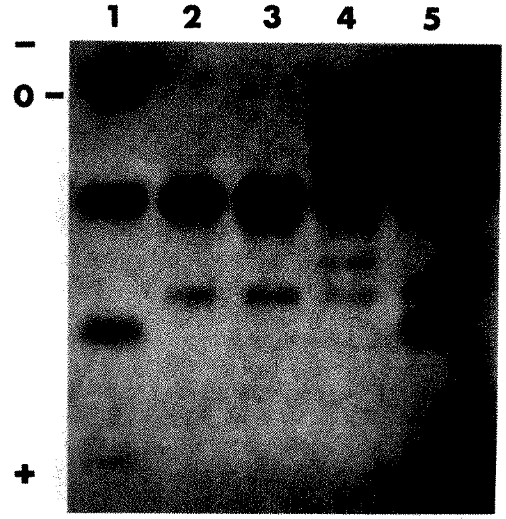

Fig. 2 Lactate dehydrogenase electrophoresis on Cellogel[13]. Cell extracts: 1, Human fibroblast; 2, mouse 3T3/TK- parental cells; 3, hybrid clone VII-2 BUdR; 4, hybrid clone VII-2 HAT; 5, mixture of extracts (1) and (2). The hybrid in channel 3 is negative and the one in channel 4 is positive for human LDH-A.

Table 1 Properties of clones used in this study

	Human chromosomes*			Human LDH-A	β Globin by Southern blot	β Globin by liquid molecular hybridisation
Chinese hamster × human	der(11)	der(15)	11			
XXI-61A	0.5	0	0	−	−	−
XXI-22A-c	0.9	0	0	−	−	−
XXI-22A-c-2b	0.8	0	0	−	−	
XXI-22A-c-2c	1.0	0	0	−	−	
XXI-22A-g	0.7	0.7	0	+	+	+
XXI-22A-g-1a	1.0	1.0	0	+	+	+
XXI-22A-g-2a	1.0	0.8	0	+	+	
XXI-22A-g-2b	0.8	0	0	−	−	−
XXI-23A-2d	1.4	0	0	−	−	−
Control DNA						
Human spleen	0	0	1.0	+	+	+
Don cell	0	0	0	−	−	−
Mouse × human		mar − 1 (11 cen → p15)	11			
VII-2 HAT		0.9	0	+	+	
VII-3 HAT		1.0	0	+	+	
VII-2 BUdR		0	0	−	−	
VII-4 BUdR		0	0	−	−	
Control DNA						
Human spleen		0	1.0	+	+	+
Mouse liver		0	0	−	−	−

*Between 15 and 30 metaphase cells per clone were analysed. Results are presented as average frequency of respective chromosome per cell.

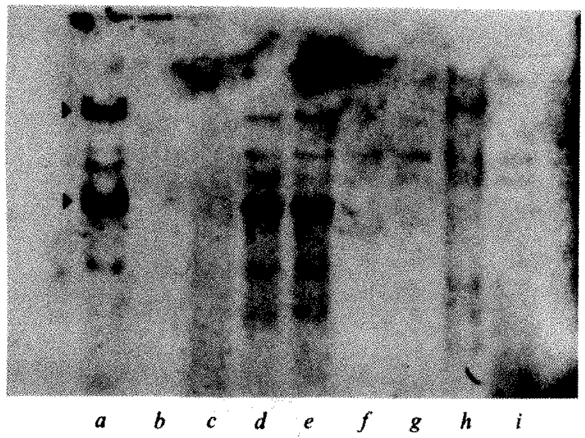

Fig. 3 Southern DNA blotting of hybrid clones and controls. Hybrid cells were collected for karyotyping, LDH-A analysis, and DNA extraction all from the same (or from a very close) passage. The cells were pelleted, and resuspended in 50 mM EDTA, 0.5% SDS, and 100 μg ml^{-1} proteinase K (Beckman). DNA extraction proceeded by addition of an equal volume of 1:1 chloroform:phenol, separation of the phases, addition of an equal volume of chloroform:isoamyl alcohol (24:1) to the aqueous phase, separation of the phases, and two ethanol precipitations. The nucleic acid was pelleted at 10,000 r.p.m. for 45 min, and resuspended in SSC (0.15M NaCl + 0.015 M Na citrate, *p*H 7.0). RNase (previously boiled for 10 min to inactivate residual DNase) was added to a concentration of 100 μg ml^{-1} and incubated at 37 °C for 1 h. Extraction with phenol:chloroform was performed, followed by a second ethanol precipitation. The DNA pellet was resuspended in deionised water and dialysed for 2 d in Tris-EDTA (100 mM Tris-HCl, *p*H 7.2, 0.5 mM EDTA). 100-μg samples of each hybrid DNA were digested for 2 h at 37 °C with *Eco*RI (Boehringer Mannheim) 2 units per μg, and electrophoresed in 1% agarose. The DNA was blotted on to nitrocellulose filters in 6 × SSC. The blots were prehybridised in 0.02% Ficoll, 0.02% polyvinylpyrollidone, 50 μg ml^{-1} denatured salmon sperm DNA, 10 μg ml^{-1} poly(A) and 0.1% SDS to prevent nonspecific hybridisation of the β-globin probe. The cDNA probe was prepared from plasmid JW102[7] which was digested with *Hha*I and separated by size on an alkaline sucrose gradient (5–20% sucrose in 10 mM Tris-HCl, *p*H 7.5, 100 mM NaCl, 1 mM EDTA). The largest fragment, which contains the β-globin insert was collected by ethanol precipitation. This fragment was then incubated in the presence of ^{32}P-dCTP, DNase and *E. coli Pol*I for nick translation[3,14]. The DNA was extracted with phenol, collected in the void volume peak on a Sephadex G-50 column, and precipitated with ethanol. The resulting double-stranded probe had a specific activity of approximately 2–4 × 10^8 c.p.m. μg and was used for the Southern blotting procedure[6]. The lyophilised cDNA probe was resuspended in the prehybridisation solution described above, boiled to denature it, and added to the nitrocellulose filters in a Scotchpak bag. Hybridisation was carried out at 65 °C for 72 h. After hybridisation, the blots were washed in several changes of prewarmed SSC, and autoradiographed with intensifying screens at −80 °C for 48 h. Upper and lower arrows indicate *Eco*RI human β-globin fragments of 6.2 and 4.0 kilobases, respectively. These correspond to the 5.2 and 3.2 kilobase fragments reported by Lawn *et al.*[15]. The large amount of DNA loaded on the gel causes these fragments to migrate more slowly. *a*, Human spleen DNA; *b*, XXI 61A; *c*, XXI 23A-2d; *d*, VII-2 HAT; *e*, VII-3 HAT; *f*, VII-2 BrdU; g, VII-4 BrdU; *h*, Don cell DNA; and *i*, mouse liver DNA.

(Fig. 2). DNA was extracted from each cell for liquid molecular hybridisation or Southern blotting to detect human β-globin gene sequences.

All hybrid cell clones were expanded to 5 × 10^8 cells, and an aliquot of each was examined for LDH-A. The LDH-A analysis was used as a marker to monitor the presence of the short arm of HC 11. The remainder of the cell pellet was extracted for DNA as described in the legend to Fig. 3.

For Southern blotting, a 100-μg sample of each hybrid DNA was digested with *Eco*RI, electrophoresed in 1% agarose, blotted on to nitrocellulose filters and annealed in stringent conditions to ^{32}P-labelled pure human β-globin cDNA (obtained from the recombinant cDNA plasmid JW102). Thirteen hybrid clones were analysed (Fig. 3; Table 1). Two bands which hybridised strongly to human β-globin cDNA were present in the human spleen control DNA (Fig. 3, lane *a*). Several lighter bands were present in all DNA samples including the Chinese hamster (Fig. 3, lane *h*) and mouse liver (Fig. 3, lane *i*) control DNA, and probably represent cross-reactivity of the human β-globin cDNA with other sequences. The five clones positive for LDH-A were also positive for the human β-globin gene (Fig. 3, lanes *d*, *e*; Table 1); the eight clones negative for LDH-A were also negative for the human β-globin gene (Fig. 3, lanes *b*, *c*, *f*, *g*; Table 1).

Liquid molecular hybridisation was carried out, as described previously[3], on six hybrid clones (Table 1, Fig. 4). Two were positive for LDH-A and the human β-globin gene; four were negative for both.

Thus, in both the Southern blot and the liquid molecular hybridisation assays, those hybrids containing the short arm of HC 11 and expressing human LDH-A were positive for the presence of the human β-globin gene, while those clones containing only the long arm of HC 11 were negative for the β-globin gene. Since p15 is the breakage point of mar-1, and 11p11 the breakpoint in the t(11; 15) translocation, the β-globin gene complex can be limited to region 11p11 → 11p15 of the human chromosome.

These mapping studies may be of significance in the study of regulatory phenomena involving the γ-δ-β globin loci. Since in mar-1 the short arm of HC 11 (containing the β-globin gene region) is attached to HC 17 (containing thymidine kinase) this

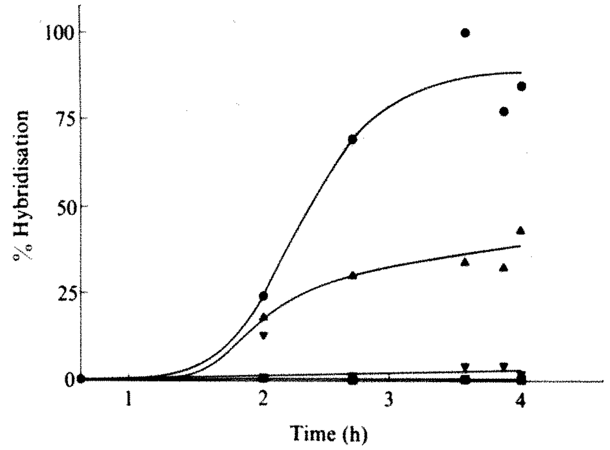

Fig. 4 Liquid molecular hybridisation of hybrid cell DNA to ^{32}P-labelled human β-globin cDNA. Hybrid cells were pelleted and extracted for DNA as described previously[3]. The double-stranded ^{32}P-labelled β-globin cDNA probe, prepared as described in the legend to Fig. 3, was further purified by hybridisation to adult human reticulocyte mRNA in 50% formamide and 3 × SSC at 66.5 °C. This temperature prohibits DNA duplex formation but allows DNA·mRNA hybridisation, which then protects the antimessage strand from subsequent digestion with S$_1$ nuclease. After addition of *E. coli* carrier DNA and alkaline buffer, the duplex was separated on a 5–30% alkaline sucrose gradient. The hot peak of 500–800 nucleotide material was pooled, neutralised, and precipitated in ethanol. The precipitate was dissolved in deionised water and desalted over a Sephadex G-25 column. The purity of the ^{32}P-labelled, single-stranded β-globin specific cDNA was tested by annealing to probe at increasing concentrations of adult human reticulocyte mRNA. For detection of the human β-globin gene in hybrid DNA, the sonicated DNA of each cell line was lyophilised in 3-mg aliquots and mixed with a cocktail of 50% formamide, 3 × SSC, and ^{32}P-labelled probe. The reaction was divided into 20-μl aliquots and sealed in glass capillary tubes coated with *E. coli* DNA to minimise sticking. All reactions were boiled for 15 min to denature the DNA and allowed to reanneal at 52.5 °C. Time points were taken from 10 min to 100 h to generate the C_0t curve. All time point samples were divided, and one-half of each digested with S_1 nuclease to determine the per cent hybridisation of each sample. (●) Human spleen DNA; (▲) XXI 22A-g; (▼) XXI 22A-g-2b; (■) Don cell DNA.

translocation product may be selectively retained in cell hybrids maintained in HAT medium. Fusion of VII-2 HAT with TK⁻ 2S mouse erythroleukaemia cells[3] should result in cell hybrids in which the human β-globin gene is maintained in an established mouse erythroid tissue culture cell line. Studies are now underway to obtain this proposed hybrid line.

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Satellite DNA is transcribed on lampbrush chromosomes

Jennifer M. Varley, Herbert C. Macgregor & Harry P. Erba

Department of Zoology, University of Leicester,
Leicester LE1 7RH, UK

During the lampbrush stage of oogenesis there is widespread transcription, and it has been estimated that the total amount of DNA transcribed may be an order of magnitude greater than that required to produce the necessary functional RNA for the oocyte[1]. We therefore considered it likely that some of the transcribed sequences have little, if any, translational significance, and may include both middle repetitive[2] and highly repeated, or satellite, sequences. Satellite DNA is generally defined as rapidly reannealing DNA which has a short basic sequence that is repeated millions of times in the genome, usually in tandem arrays[3,4]. The short repeated length, coupled with the organisation of satellite sequences in high order molecular weight tandem arrays in heterochromatic regions, have been put forward as reasons for supposing that this type of DNA is not normally transcribed[5]. We report here that we have looked for and found evidence of transcription of satellite DNA on lampbrush loops in oocytes of the crested newt, *Triturus cristatus carnifex*.

The technique that we have used to detect transcription is that of DNA/RNA-transcript hybridisation[6], where a radioactively labelled DNA probe is hybridised to nascent RNA transcripts on the lampbrush loop. Preliminary studies using this approach[7] indicated that highly repeated DNA sequences are transcribed around some lampbrush loops during oogenesis in *T.c. carnifex*. However, the probe used in these experiments undoubtedly

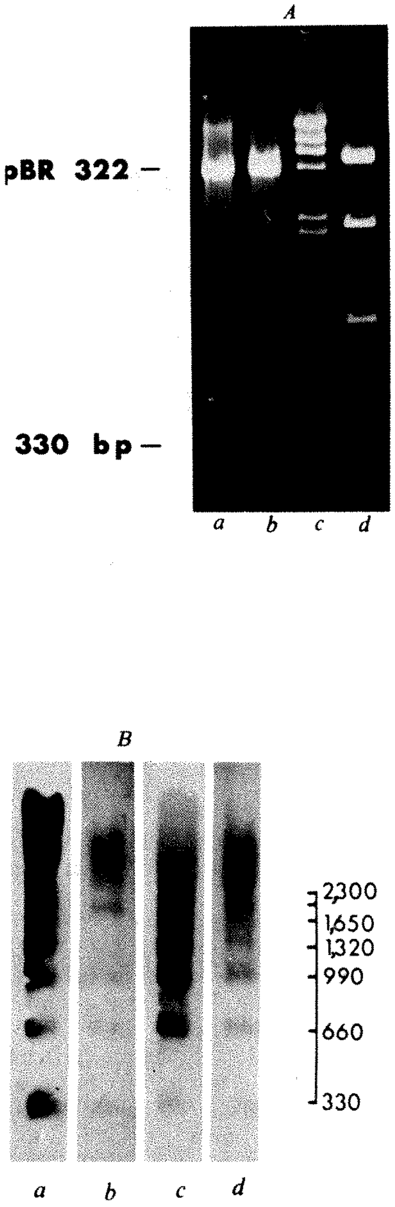

Fig. 1 *A*, Analysis of recombinant plasmids pTcS1 and pTcS2 by restriction endonuclease cleavage and electrophoresis on a 1% agarose gel. pTcS1 and pTcS2 were digested with *Bam*HI (tracks *a*, *b*). Linear pBR322 and a 330-base pair fragment are resolved as indicated. Tracks *c* and *d* show *Hin*dIII digests of λ and PM2, respectively. pTcS1 and pTcS2 had been produced by ligating pBR322 cleaved with *Bam*HI and the partially purified *Bam*HI 330-base pair satellite sequence. Clones containing plasmids that failed to confer tetracycline resistance were grown in M9 medium containing 100 μg ml^{-1} ampicillin at 37 °C. The plasmids were amplified using chloramphenicol (250 μg ml^{-1})[18] and covalently closed circular plasmid DNA was isolated as supercoils by lysozyme-Brij 58-Na deoxycholate lysis of the cells[19] followed by centrifugation in caesium chloride–ethidium bromide. Plasmids containing *T.c. carnifex* DNA were propagated under P2 containment as defined by the NIH. *B*, Autoradiographs of nitrocellulose filters which have been hybridised to pTcS1 and pTcS2 nick translated with [α-^{32}P]dCTP (Radiochemical Centre; Code no. PB 205; specific activity 2–3,000 Ci mmol^{-1}). Tracks *a* and *c* represent transfers of total *T.c. carnifex* DNA digested with *Bam*HI, tracks *b* and *d* represent total *T.c. carnifex* DNA digested with *Kpn*I, which also cleaves once within this 330-base pair repeat. Tracks *a* and *b* have been hybridised with pTcS1, tracks *c* and *d* with pTcS2. In each case, a ladder of sequences representing multimers of the 330-base pair repeat hybridise to the cloned fragments, showing that the cloned fragment represents a tandemly repeated satellite sequence. Autoradiography was carried out for 2–5 days at −20 °C using an Ilford fast tungstate intensifying screen.

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contained some middle repetitive and unique DNA sequences, which could have been responsible for the results obtained. To determine whether satellite DNA is transcribed during oogenesis, we have isolated a 330-base pair satellite sequence from total *T.c. carnifex* DNA by digestion with the restriction endonuclease *Bam*HI. This restriction fragment was inserted into the *Bam*HI site in the tetracycline resistance gene in pBR322 (ref. 8). Plasmid DNA was purified from cultures of *Escherichia coli* K12 strain HB101 (ref. 9) containing the recombinant plasmid, and digested with *Bam*HI. After agarose gel electrophoresis, bands corresponding to linear pBR322 and to a sequence 330 base pairs long (Fig. 1*A*) were visible. To confirm that the 330-base pair sequence was a monomer of a tandemly repeated satellite, two of the recombinant clones, pTcS1 and pTcS2, were labelled *in vitro* with $[\alpha^{32}P]$dCTP. These probes were then hybridised to total *T.c. carnifex* DNA that had been digested with *Bam*HI, electrophoresed on agarose and transferred to nitrocellulose filters[10]. Both clones hybridised to a ladder of sequences corresponding to monomers and multimers of a 330-base pair sequence (Fig. 1*B*), confirming that we had cloned a tandemly repeated sequence.

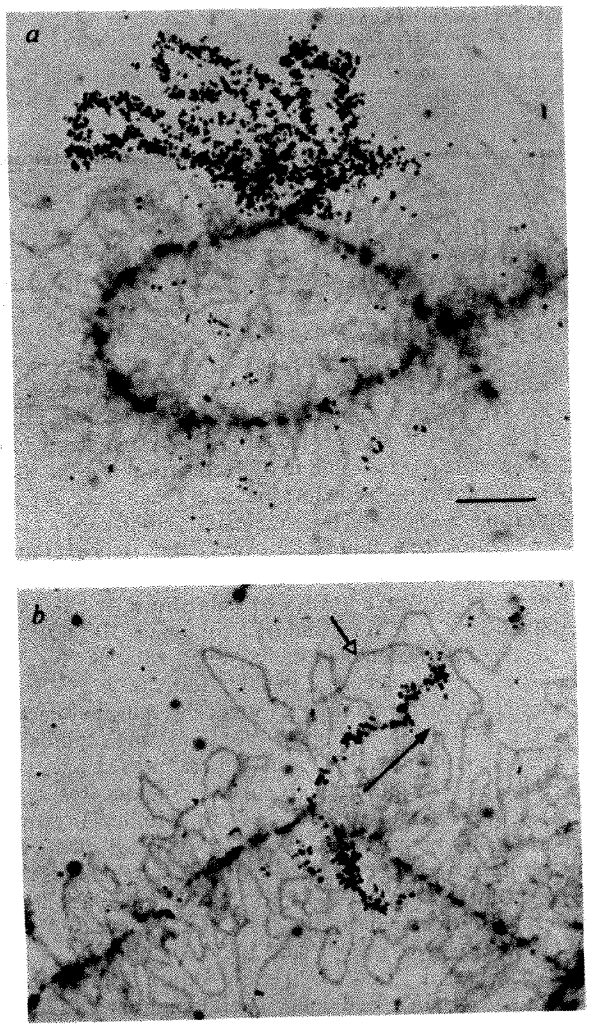

Fig. 2 Light micrographs of DNA/RNA-transcript hybrids of ^{3}H-labelled pTcS1 DNA onto lampbrush loops of *T.c. carnifex*. Lampbrush chromosomes were isolated as described previously[12], but were fixed in 70% ethanol only. Hybridisation was carried out in 50% formamide, 4 × SSC at 37 °C for 12 h (ref. 2). The specific activity of the probe was 2×10^6 c.p.m. per μg and 1–2×10^5 c.p.m. were used per preparation. The autoradiographic exposure time was 2–4 days. Scale bar, 10 μm. *a*, Heavily labelled multiple loops at 8 units (ref. 16) on the heteromorphic long arm (HTA) of chromosome I. The level of labelling is very high over the whole length of the loop. *b*, Partially labelled loop pair at 54 units (ref. 16) on the HTA of chromosome I. There is a distinct gradient in the amount of silver grains, indicating that transcription proceeds in the direction shown (arrow). The unlabelled region can also be seen (open arrow).

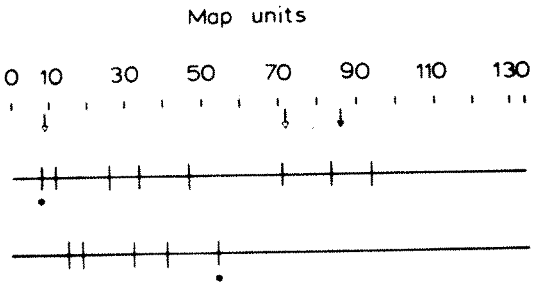

Fig. 3 Diagrammatic representation of the loops labelling on chromosome I of *T.c. carnifex* using pTcS1 to hybridise to the nascent RNA transcripts. The numbers associated with each loop give the distance from the left end of the chromosome in terms of units described by Callan and Lloyd[16]. The labelled loop pairs are drawn as bars on a line that represents the entire length of chromosome I. The centromere is at 86 units (arrow), and the heteromorphic region (HTA) lies between 9 and 72 units (open arrows). The top line represents the longer HTA, the bottom line the shorter HTA. The two loop pairs marked with asterisks are those at 8 units on the long HTA and at 54 units on the shorter HTA that are shown in Fig. 2. Conditions of hybridisation were as described in Fig. 2 legend.

DNA from clone pTcS1 was then nick translated with ^{3}H-labelled triphosphates[11] and used as a probe in DNA/RNA transcript hybrids onto lampbrush loops from *T.c. carnifex*[12]. After a 72-h autoradiographic exposure, several loops were heavily and specifically labelled. Most of these loops were situated on the long arm of chromosome I (Figs 2, 3). Results using the cloned satellite sequence confirm and extend results obtained using highly repeated DNA isolated by renaturation kinetics, by caesium salt centrifugation and by isolation of restriction fragments from agarose gels[13]. The level of labelling over some of the loops, and the short autoradiographic exposure needed, indicate that in some cases, the loop RNA consists almost exclusively of satellite DNA transcripts (Fig. 2*a*).

Ribonuclease treatment of lampbrush chromosome preparations (2 h at 37 °C in a solution of 100 μg ml^{-1} ribonuclease A and 1,000 units ml^{-1} ribonuclease T1 in 1 × SSC) before incubation with labelled pTcS1 completely eliminated *in situ* hybridisation. No part of any lampbrush chromosome or any other structure became labelled in our autoradiographs, even after exposures of several weeks. Pretreatment of preparations with deoxyribonuclease 1 left the level of *in situ* hybridisation with pTcS1 unimpaired. Labelled, alkali-denatured pBR322 DNA was also used as a control, using precisely the *in situ* hybridisation and washing conditions as for pTcS1. Autoradiographs of pBR322 preparations were completely negative.

There are two important aspects to this study. The first is that we consider that our data provide the first clear evidence that DNA belonging to the satellite class is transcribed during the lampbrush stage of oogenesis in *T.c. carnifex*. The second is related to the unusual structure of the long arm of chromosome I in this species, and will be discussed later.

We consider it unlikely that the RNA transcribed from a satellite sequence will be translated. If it were, then any polypeptide produced would be very unusual. However, the question that remains is why satellites should be transcribed at all during the lampbrush stage of oogenesis. One possibility is that the RNA transcripts of satellite sequences in some way regulate the expression of other, possibly translatable, sequences in the germinal vesicle. Such control would be stage specific, and studies on this problem are in progress in our laboratory. A second explanation might be that transcription of satellite sequences in the germinal vesicle is incidental, and that on a lampbrush loop the RNA polymerases overrun normal termination signals and read right through stretches of satellite DNA. The majority of the highly repeated DNA in *T.c. carnifex* is clustered in pericentric regions, and on the long arm of chromosome I (refs 7, 13, 14). If transcription does overrun normal termination signals, satellite DNA is more likely to be transcribed on chromosome I simply because there is so much of it in this region.

The presence of highly repeated DNA on the long arm of chromosome I correlates in an unknown way with structural heteromorphism and a lack of chiasma formation in this chromosome arm in *Triturus cristatus*[14–17]. We have evidence that the distribution of the satellite sequences on the two long arms of chromosome I may be different[13]. We do not know why such a situation should exist, what evolutionary significance it may have, or why satellite DNA should be transcribed on this unusual chromosome.

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Oocytes in the testis

Anne McLaren

MRC Mammalian Development Unit, Wolfson House,
4 Stephenson Way, London NW1 2HE, UK

In normal sexual differentiation, XX germ cells develop into oocytes in an ovary, and XY germ cells undergo spermatogenesis in a testis. In some genetic abnormalities of sexual differentiation, and other abnormal situations such as experimental mouse chimaeras, XX germ cells may be present in a male embryo[1,2]. They will still migrate into the genital ridges, but in the environment of a testis they usually degenerate at or before the onset of meiosis. Whether they pursue a male or a female pathway of development before degeneration is not yet established. Very occasionally growing oocytes have been observed within seminiferous tubules, for example in a 16-yr-old hermaphrodite who was brought up as a girl and only came to medical attention because he wished to be recognised as a boy[3]. Such tubules usually form part of an ovotestis (refs 3–5 and P. S. Burgoyne, personal communication), though large numbers of primary and growing oocytes have also been observed in apparently normal testes of two mouse chimaeras, one an 8-d-old bilateral hermaphrodite (M. Spiegelman, personal communication) and the other a 5-d-old male[6]. The phenomenon is usually confined to very young animals, apart from one adult mouse chimaera (P. S. Burgoyne, personal communication) in which the tubules were disorganised and the oocytes that they contained were hyalinised, as though they had undergone atresia. In most cases (refs 3, 5, 6 and P. S. Burgoyne and M. Spiegelman, personal communications) the individuals were known to possess both an XX and an XY cell population, and the chromosomal sex of the oocytes was not established. In contrast, I report here that a substantial proportion of genetically sex-reversed male mice, in which all cells are initially XX in chromosome constitution, contained healthy growing oocytes within their testes up to at least 15 days of age.

The autosomal gene *Sex-reversed* (*Sxr*)[7] causes genetically female (XX) mice to develop as phenotypic males. Such *Sxr*/+ XX males are sterile, as the XX germ cells are unable to undergo spermatogenesis. Apart from the absence of germ cells, the testes are grossly normal in morphology. Cattanach *et al.*[7] examined some testes of sex-reversed mice in the prenatal and early postnatal period, and concluded that the XX germ cells, although present initially, are lost by 10 days of age.

During a further study of the testes of prepubertal *Sxr*/+ XX mice, I examined serial sections of 21 testes from sex-reversed mice, heterozygous for X-linked coat-colour markers and killed between 8 and 18 days of age. Six proved to contain growing oocytes. No oocytes were found in 30 testes from normal male sibs in the same age range. The fact that Cattanach *et al.*[7] did not detect any growing oocytes in their material is not in conflict with the present finding, for within this age range they only examined two animals (at 10 days of age).

Three of the sex-reversed testes contained one oocyte each, one contained two, one nine, and one contained 100 oocytes. The oocytes were located within the testis tubules (Fig. 1), usually in the neighbourhood of the rete testis (Fig. 1*c*). Each was surrounded by an incomplete layer of cells, morphologically similar to the Sertoli cells lining the tubules, though somewhat more flattened, and resembling in general appearance the incomplete layer of granulosa cells surrounding oocytes in the mouse ovary at birth. No sign was seen of the follicular growth that begins in the mouse ovary about 3 days after birth. The oocytes themselves, on the other hand, had enlarged considerably since birth, measuring 30–50 μm in diameter, about the same size as ovarian oocytes of the same age. Thus at this stage of development, oocyte growth and follicular growth are not necessarily associated. The state of preservation of the oocytes varied from good, with a well defined cell membrane, an intact nuclear membrane, and a distinct nucleolus, to very degenerate. In one case the nuclear material was represented by a large number (more than 100) of very small chromosome-like bodies (Fig. 1*d*), reminiscent of the highly condensed chromosomes seen in cells blocked in metaphase (see, for example, Paterson's illustration of air-dried preparations of dying embryos homozygous for the gene *oligosyndactyly*[8]).

Twelve adult (6–9 weeks) *Sxr*/+ XX testes were also serially sectioned, but none contained oocytes (0/12 compared with 6/21; $P < 0.05$, by Fisher's exact test). This suggests that all the oocytes would have degenerated before sexual maturity was reached. Perhaps such oocytes are unable to survive the changes in the testicular environment that must occur between 1 and 2 weeks after birth in the normal male mouse, to induce the entry of XY germ cells into meiosis.

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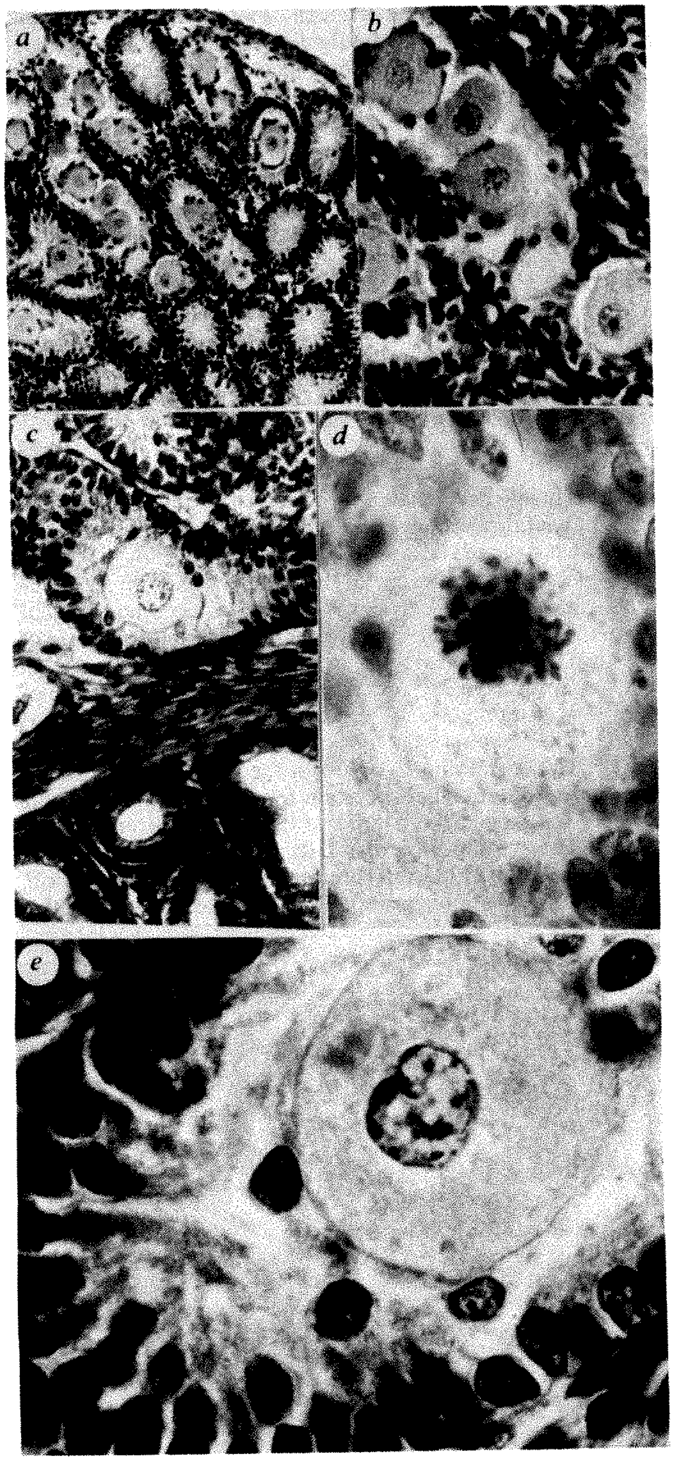

Fig. 1 *a*, Testis of 11-d-old XX *Sxr*/+ male, showing numerous growing oocytes within the intact seminiferous tubules. *b*, Higher power view of some of the tubules shown in (*a*). The oocytes are surrounded by an incomplete layer of flattened follicle-like cells. *c*, Single oocyte in tubule of 10-d-old XX *Sxr*/+ male, adjacent to rete testis. *d*, Degenerate oocyte, from 11-d-old XX *Sxr*/+ testis. Nucleus appears to have broken down into numerous small chromosome-like bodies. *e*, Growing oocyte, with intact cell membrane and nucleus, from 15-d-old XX *Sxr*/+ testis.

Does the fact that the XX germ cells in the sex-reversed testes were undergoing oogenesis, as befits their chromosomal sex, imply that they were developing autonomously? The testes of XX/XY mouse chimaeras contain initially both XX and XY germ cells. A study of such testes during the prenatal period[9] suggested that XX but not XY germ cells entered meiotic prophase before birth, at the normal time for XX germ cells undergoing oogenesis in the ovary. XY germ cells can occasionally be induced to enter meiosis prematurely[10], but apparently only if their environment is altered, for example, by transplantation beneath the kidney capsule[11], or explantation *in vitro*[12]. In the rare case of an XX↔XY chimaera gonad differentiating as an ovary, XY germ cells have been reported to undergo oogenesis; one gave rise to a functional egg[13,14]. XO germ cells, which resemble XY germ cells in having only one X chromosome, are capable of undergoing spermatogenesis if they are located in a testis, for example in an *Sxr*/+ mouse, but the spermatozoa that they form are abnormal and show little or no motility[7,15]. The few spermatogonia reported by Cattanach *et al.*[7] in the 2-d, 5-d and 6-d-old *Sxr*/+ XX testes that they examined, might therefore have been XO in chromosome constitution, as were the spermatogenic cells isolated from the occasional tubule seen to be supporting active spermatogenesis in adult *Sxr*/+XX males[15]. Alternatively, if the spermatogonia in the newborn testes were XX, it would suggest that some XX germ cells can take a male developmental pathway, others a female pathway.

If this were so, development cannot be autonomous, but must be under some environmental influence. H–Y antigen, thought to have a male-determining role in the differentiation of the testis[16,17], seems more likely to act on the somatic tissues, inducing the formation of the testis tubules, than on the germ cells. The location of the oocytes in *Sxr*/+ XX testes near the rete testis lends some support to the postulate of Byskov, that entry of germ cells into meiosis before birth is under the control of a diffusible meiosis-inducing substance (MIS)[12] produced by the mesonephric rete region in both female and male fetuses[18]. According to this postulate, germ cells in the testis would normally be protected from the effects of MIS by their location within the tubules, where a hypothetical meiosis-preventing substance, secreted perhaps by the Sertoli cells, would be present in high concentration[19]. The observations on *Sxr*/+XX germ cells reported here, as well as previous observations on XX↔XY chimaeras, would imply that germ cells with two X chromosomes are more susceptible to the meiosis-inducing influence of MIS than are germ cells with only one X chromosome, and hence are more likely to undergo oogenesis. Perhaps the germ cells that Cattanach *et al.*[7] described as spermatogonia in the newborn *Sxr*/+XX mice were those located further from the rete, that failed to come under the influence of MIS and hence started development along the male pathway.

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Sequence-dependent variations in the backbone geometry of a synthetic DNA fibre

Heisaburo Shindo & Steven B. Zimmerman

National Institute of Arthritis, Metabolism and Digestive Diseases,
National Institutes of Health, Bethesda, Maryland 20205

There are many examples of proteins which interact with specific DNA sequences (see references cited in refs 1 and 2). It is not clear whether specificity is derived from proteins sensing directly the base sequence of the DNA or is a result of unique secondary or tertiary structural features induced by certain DNA sequences. Recent studies have indicated the occurrence of several types of symmetries and sequence irregularities in DNA regions which interact with regulatory proteins, but they do not provide information on three-dimensional structure[3]. The Watson–Crick base-paired double helix can be observed in different conformations[4–6], depending on humidity and salt concentration. Within the limited resolution of X-ray fibre diffraction at high relative humidity and in the appropriate salt conditions, studies indicate that natural DNA as well as synthetic repeating polynucleotide duplexes adopt essentially the same average secondary structure, namely the B conformation[7]. This implies that all residues adopt a single backbone conformation. Recently, however, a high-resolution proton NMR study[8] has demonstrated that a change from a regular conformation may occur at the junction region of block copolymers. ^{31}P NMR studies strongly suggest that alternating polynucleotides can have alternating backbone conformations in solution[9,10]. Klug *et al.*[11] have proposed an alternating structure for poly(dAdT)·poly(dAdT), based on a single crystal X-ray diffraction study of the tetranucleotide, dA-dT-dA-dT (ref. 12). We report here structural ^{31}P NMR studies which show the existence of such variation in the fibre form of a synthetic DNA, poly(dAdT)·poly(dAdT). This confirms our earlier conclusion[13] that natural DNA has a non-uniform backbone conformation.

Figure 1 shows an X-ray diffraction image from a poly(dAdT)·poly(dAdT) fibre at high relative humidity; the pattern is similar to the B form patterns reported by Davies and Baldwin[14]. From this image, the pitch and the rise per residue were found to be 32.3 and 3.3 Å, respectively, corresponding to 9.8 residues per turn. This implies that the alternating copolymer is in the B conformation in these conditions.

Figure 2 compares the ^{31}P NMR spectra at 24.3 MHz from natural DNA fibres and poly(dAdT)·poly(dAdT) fibres at two different fibre orientations relative to the magnetic field, H_0. Observed NMR parameters are shown in Table 1. Strikingly, poly(dAdT)·poly(dAdT) fibres when orientated parallel to the magnetic field exhibit two well defined resonances, whereas natural DNA exhibits a single broad resonance in the same conditions (Fig. 2). The former result is consistent with the observation of a doublet in the ^{31}P spectrum for a 145 base-pair poly(dAdT)·poly(dAdT) fragment in solution[10]. As the two resonances occur in nearly equal intensity, these results strongly imply an alternating conformation of the phosphodiester groups of DNA both in solution and in the solid state, stemming from the alternating base sequence of this polymer. As discussed previously[13], the line width of the parallel spectrum is partly a result of imperfect orientation of the molecules relative to the fibre axis. We observed two resonances with a separation of 21 p.p.m. in the parallel spectrum for poly(dAdT)·poly(dAdT). The total line width is 39 p.p.m., of which instrumental factors and magnetic susceptibility may account for ≤5 p.p.m. Thus, the individual line width can be estimated to be about 13 p.p.m. (that is, 39 − 5 − 21 p.p.m.). This estimated value corresponds to that calculated for a 4–6 degree deviation of the helical axis about the long axis of the fibre[13]. In other words, the observed individual line width for poly(dAdT)·poly(dAdT) can be explained by a small amount of molecular disorientation in the fibre. Applying similar reasoning to the natural DNA spectra, the distribution of chemical shifts extends over 24 p.p.m. (that is, 42 − 5 − 13 p.p.m.), which we take as a reflection of the limit of variation in the orientation of the phosphodiester group in DNA with heterogeneous sequences. The deviation in the orientation of the phosphodiester group in natural DNA was previously estimated to be of the order of ±20° from the average orientation about any of the individual orthogonal axes[13]. Note that the extremes of this range may be related to ribose ring puckering[12], but do not necessarily imply large changes either in base stacking conformations or in individual dihedral angles of the backbone. Thus, we conclude that irregularity in the backbone conformation is an ubiquitious phenomenon not restricted to a small region of DNA; presumably the irregularity is sequence-dependent. This irregularity of phosphodiester conformation is, of course, highly accentuated in poly(dAdT)·poly(dAdT). The high affinity of the *lac* repressor for this duplex[15–18] may depend on just such structural characteristics.

The average chemical shift of the parallel spectrum for natural DNA occurs between the two resonances of poly(dAdT)·poly(dAdT). This implies that the conformations of the phosphate group in ApT and TpA sequences deviate in opposite senses from its average conformation in the B form of DNA. It is of interest that the one resonance, which is shifted downfield by 12 p.p.m. from the peak of the parallel spectrum of natural DNA (see Table I), occurs at the position of the maximum peak of the parallel spectrum for the A form of natural DNA[13]. This may suggest that the orientation of one of the phosphodiester groups is similar to its orientation in the A form of DNA. One plausible interpretation for the chemical shift changes is that the ionic group, O=P—O⁻, of the one of the

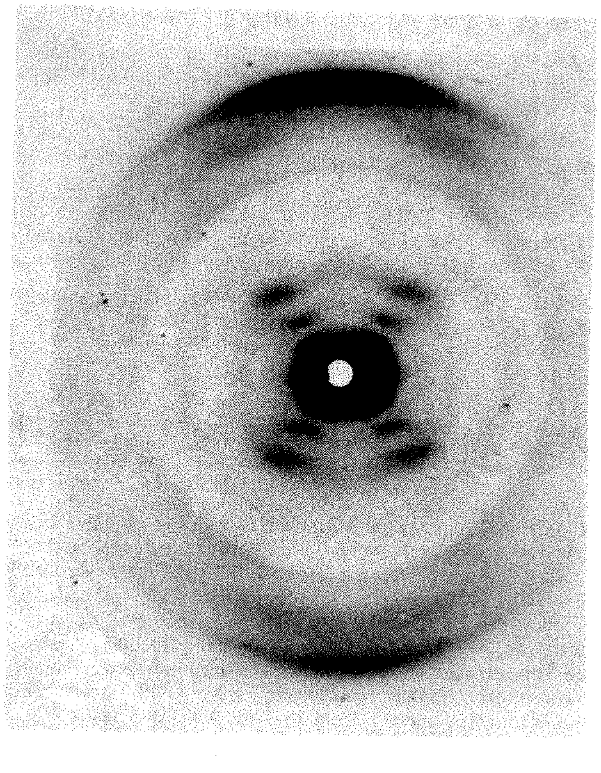

Fig. 1 X-ray diffraction photograph from a poly(dAdT)·poly(dAdT) fibre at room temperature and 98% relative humidity. The alternating copolymer (P. L. Biochemicals) was dissolved in a small amount of 10 mM NaCl, heated in a boiling water bath for about 1 min, and then cooled slowly to room temperature. The fibres were formed at 4 °C; otherwise their preparation and the collection of the diffraction pattern are as described previously[20].

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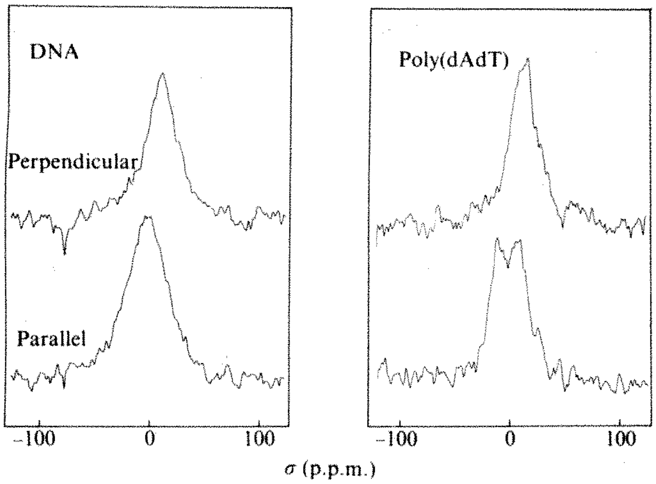

Fig. 2 The proton dipolar decoupled and cross-polarised ^{31}P NMR spectra (24.3 MHz) at 25 °C from poly(dAdT)·poly(dAdT) fibres (right) and salmon sperm DNA fibres (left). The top spectra for both DNAs were obtained from perpendicular-orientated fibres (relative to the magnetic field) and the bottom spectra from the parallel-orientated fibres. The method of controlling humidity and instrumentation were as described previously[13]. Measurement conditions; spectral window ± 20 kHz, data points 1,024 real, cross-polarisation time 1.5 ms, delay time 2 s, line broadening 50 Hz, scan numbers 21,000 for the DNA and 70,000–80,000 for the synthetic copolymer.

phosphates is pushed out on the surface of the duplex while the other is pushed into the major groove of the DNA, as judged from the general features shown by phosphate groups in the B and A form structures, respectively[4,6,7]. Therefore, the characteristic cleavage of the P—O(3′) bond of adenosine in poly(dAdT)·poly(dAdT) by pancreatic DNase I (ref. 19) may not actually be sequence-specific in a rigorous sense but rather the result of the difference in its accessibility to the enzyme. As a consequence, the upfield resonance is tentatively assigned to the phosphate of the ApT sequences and the downfield resonance to the phosphate in the TpA sequences.

Finally, we will consider the spectra obtained when fibres are orientated perpendicular to the applied magnetic field. To account for the perpendicular spectra, reorientational motion about the helical axis must be considered[13]. In principle, the perpendicular spectrum should display a doublet, assuming that the molecules are rigid and that all phosphodiester conformations are symmetrical with respect to the helical axis. The perpendicular spectrum for natural DNA exhibits a singlet (Fig. 2), which was interpreted previously in terms of rotational motion about the helical axis[13]. Furthermore, the line width of the parallel spectrum is broader by 10 p.p.m. than that of the perpendicular spectrum for this DNA as a result of the variation in phosphodiester orientation. The same considerations may be applied to the alternating polynucleotide. A rather asymmetrical singlet was observed (Fig. 2), whereas a doublet would be expected even with rapid rotational motion about the helical axis. By using the estimated individual line width (13 p.p.m.) the separation of the resonances, if any, for the perpendicular spectrum would be about 10 p.p.m. or less, so that the doublet appears as a broad singlet at the frequency used here, but would possibly be split at higher frequencies. The structural considerations from the NMR data on the phosphodiester group in this synthetic polynucleotide will be discussed in more detail elsewhere.

Table 1 ^{31}P NMR parameters obtained at 24.3 MHz from salmon sperm DNA and poly(dAdT)·poly(dAdT) fibres at different orientations to the magnetic field

	RH (%)	Rotation angle (deg)*	Chemical shift† σ (p.p.m.)	Line width (p.p.m.)
Na-poly(dAdT)·poly(dAdT)	98	0	−17.1 4.0	39
		90	5–13	29
Na-DNA‡	98	0	−5	42
		90	8	32

* Rotation angle of zero corresponds to a parallel orientation of the fibres to the magnetic field and 90° to a perpendicular orientation.

† Measured from trimethylphosphate as external reference; upfield is positive.

‡ Data from ref. 13.

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X-ray structure of the ternary complex Zn(II)-ATP-2,2′-bipyridyl and possible model for ATP transport

P. Orioli*, R. Cini*, D. Donati† & S. Mangani*

* Istituto di Chimica Generale,
† Istituto di Chimica Organica dell'Università di Siena, Siena, Italy

Ternary complexes between metal ions, nucleotides and aromatic heterocyclic amines are being investigated by X-ray diffraction as possible models for enzyme–metal–substrate interactions[1,2]. Kennard and coworkers[3] have reported the crystal structure of the ATP disodium salt. Although more recently several crystal structures of binary and ternary complexes between metal ions and nucleoside monophosphates have been reported, no structure combining metal complexes with nucleoside di- or triphosphates has been reported. Most divalent metal ions catalyse the nonenzymatic transfer of phosphate from nucleoside polyphosphates to various acceptors[4]. Therefore, these compounds in water solution undergo extensive hydrolysis in the presence of metal ions, leading to the formation of the monophosphates; for instance, at *p*H 6.5, Cu^{2+} accelerates the hydrolysis of ATP by a factor of about 300 (ref. 5). We report here the crystal and molecular structure of the complex $[Zn(II)\text{-}H_2ATP\text{-}2,2'\text{-bipyridyl}]_2\cdot 4H_2O$, determined by single crystal X-ray analysis. The complex molecule provides a possible model for ATP transport and phosphate group transfer mechanism.

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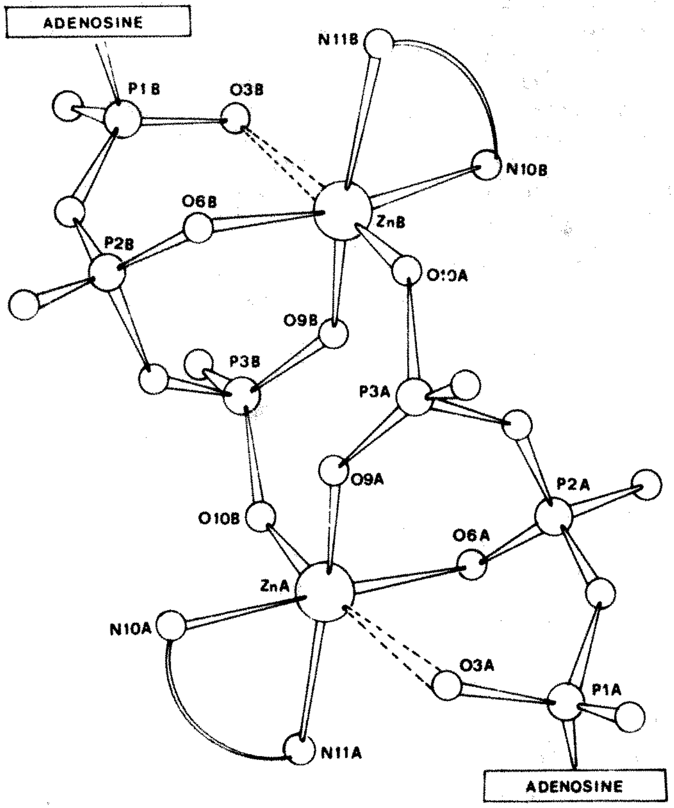

Fig. 1 Schematic drawing of the molecule, showing the mode of bonding of ATP.

Sigel and coworkers have shown that the presence of a second ligand possessing stacking capabilities (bipyridyl, phenantroline, tryptophan) greatly reduces the metal ion promoted dephosphorylation[6]. The inactivity towards hydrolysis is due to the formation of very stable ternary complexes between the nucleoside triphosphate, the metal ion and the second ligand. Stacking interactions between the heterocyclic base and the purine or pyrimidine moieties enhance the stability of the ternary complex[7].

We have been able to isolate microcrystalline compounds between ATP, 2,2′-bipyridyl and some 3d metal ions, such as Mn(II), Co(II), Cu(II), and Zn(II) in 1:1:1 ratio. These compounds are readily formed on mixing hydroalcoholic solution of Na_2H_2ATP and 2,2′-bipyridyl with an aqueous solution of the metal sulphates in equimolar ratio. On the basis of the analytical and X-ray results, the complexes can be formulated as $[M\text{-}H_2ATP\text{-}2,2'\text{-bipyridyl}]_2\cdot 4H_2O$. The solid compounds formed are essentially insoluble in most common solvents and only slightly soluble in water. Their X-ray powder patterns are very similar and only slightly soluble in water. Their X-ray powder patterns are very similar, minor differences being noted for the copper complex. Very small crystals, only for the zinc compound, were obtained on mixing dilute solutions of the reactants at *p*H 4. Trials to grow larger crystals failed, owing to the precipitation of hydrolysis products upon the crystals on standing.

X-ray diffractometer data were collected on one of these crystals with the following results: $a = 11.105(3)$, $b = 25.223(7)$, $c = 10.539(3)$ Å, $\beta = 91.34(4)°$, monoclinic, space group $P2_1$, with two dimeric units in the cell. Patterson and Fourier syntheses gave readily all the atomic positions, which were subsequently refined by least squares methods to an *R* factor of 9.8% over 1,617 reflections with intensity greater than twice their standard deviation.

The structure of the compound consists of dimeric molecules in which two zinc atoms are held together by two —OPO— bridges from the γ-phosphate groups of two ATP molecules, an arrangement which leads to the formation of an eight-membered puckered ring (Fig. 1). In the dimer both zinc atoms show a distorted octahedral coordination, formed by two oxygen atoms from different γ-phosphate groups, one oxygen atom from the β-phosphate group and the two nitrogen atoms of the bipyridyl ligand. The sixth position is completed by an α-phosphate oxygen atom which is only weakly bound, the ZnA–03A and ZnB–03B distances being respectively 2.70(4) and 2.42(4) Å. The other Zn–O distances average 2.02(3) Å and the mean Zn–N distance is 2.15(4) Å.

The structure is held together by strong intermolecular stacking interactions between bipyridyl and purine groups, and by

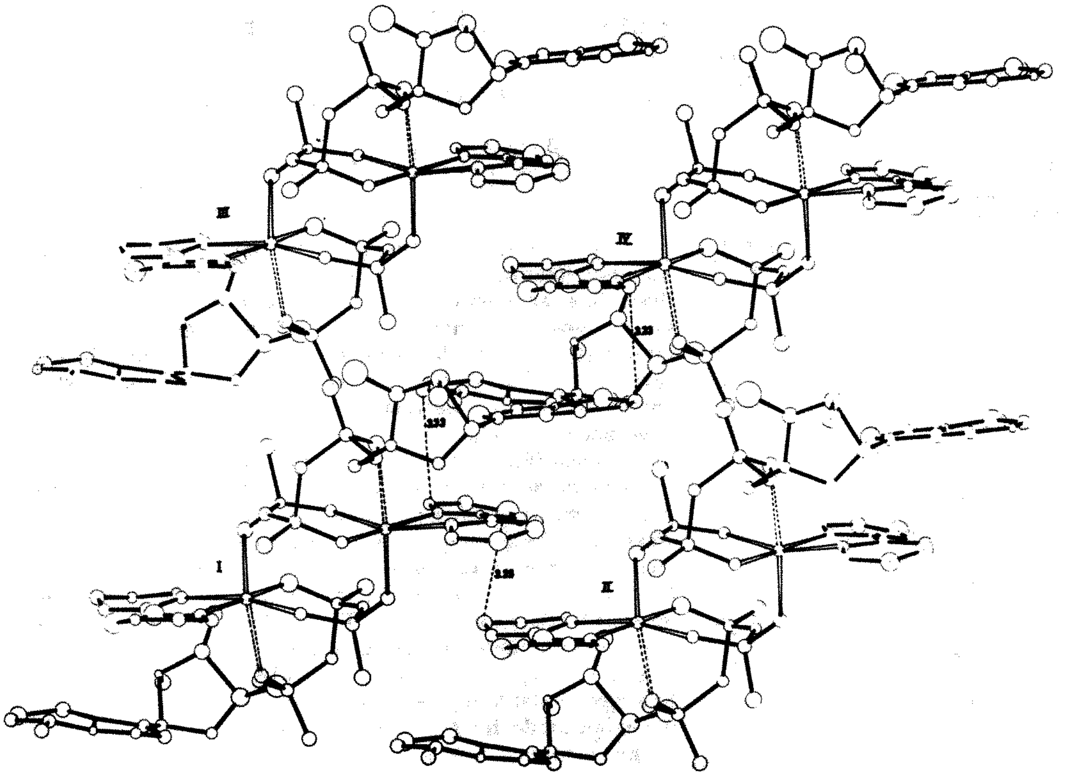

Fig. 2 Diagram of four dimeric units in $[Zn(II)\text{-}H_2ATP\text{-}2,2'\text{-bipyridyl}]_2\cdot 4H_2O$ showing the intermolecular stacking. Water molecules have been omitted for clarity. The molecules are in the following positions: I (*X*, *Y*, *Z*); II (*X* − 1, *Y*, *Z*); III (*X*, *Y*, *Z* + 1); IV (*X* − 1, *Y*, *Z* + 1). There are two types of stacking interactions: a bipyridyl–purine interaction and a bipyridyl–bipyridyl self stacking. The stacking sequence is the following (B, bipyridyl; P, purine): $-B_b(II)-B_a(I)-P_b(IV)$; $P_a(I)-B_b(IV)-B_a(III)-$; and so forth (a, b refer to the two monomers in the same dimeric molecules). The shortest distances in the stacking sequence are also shown.

hydrogen bonds between most of the polar groups. Water molecules appear to be disordered in the lattice.

Although one must be careful in drawing conclusions from the solid state, the present structure is in agreement with the results from the solution studies concerning the stability of ATP ternary complexes towards hydrolysis[7]: (1) the chelating bipyridyl does not allow interaction of the metal ion with N7 of the base moiety; (2) binding of the phosphate chain takes place essentially through the β- and γ-phosphate groups, the interaction with the α-phosphate being rather weak. Another feature arising from the solution studies is the stability enhancement due to intramolecular stacking between purine and bipyridyl. The present structure shows very strong stacking interactions between different molecules, as expected in a crystal, where intermolecular forces are much stronger than in solution (Fig. 2).

Finally it should be pointed out that the present structure also supports the mechanism proposed for the enzyme-catalysed transfer of the phosphoryl group from ATP[7]. In fact, shift of the metal ion along the phosphate backbone into β, α coordination leading to a labile γ-phosphate group, can be simply performed by a slight shortening of the Zn–03 bond and consequent loosening of the Zn–09 bond.

Bond lengths and angles and detailed conformational analysis of the molecule as well as physicochemical characterisation of all the four complexes will be reported elsewhere.

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Sequence of a ribosomal RNA gene intron from *Tetrahymena*

Martha A. Wild*

Division of Membrane Biology and Biochemistry, Institute of
Experimental Pathology, German Cancer Research Center,
6900 Heidelberg, FRG

Reinhold Sommer

Mikrobiologie, Universität Heidelberg, 6900 Heidelberg, FRG

Recently, several genes coding for messenger RNA, transfer RNA and ribosomal RNA in eukaryotes have been found to be interrupted in their coding regions by DNA sequences which are not represented in the mature RNA transcripts (see ref. 1 for review). Many of these intervening sequences, or introns[2], are now known to be transcribed in the precursor RNA, from which they are subsequently processed out to form the mature RNA[3–10]. As the intron–exon junctions must in some way be recognised for accurate splicing, the nucleotide sequences of these regions from a number of protein-coding and tRNA genes have been analysed[11–18]. Sequence homologies were found at the splice points of the protein-coding gene introns from diverse organisms[12,13], but the tRNA intron boundaries were not similar to these[14,15]. This has led to the speculation that different splicing activities are necessary for the processing of introns in mRNA and tRNA precursors[19]. We report here the sequence of a ribosomal RNA gene intron from *Tetrahymena* in which intron–exon junctions differ from those analysed to date.

In *Tetrahymena pigmentosa*, restriction endonuclease mapping and electron microscopic analysis of R-loops[20], as well as recent S_1 nuclease mapping experiments (ref. 21 and J. Engberg, personal communication) have demonstrated that an intervening sequence is present in the macronuclear 25S ribosomal RNA genes of certain strains, but is absent from the rDNA in others. All the rDNA molecules of each strain are of one kind, either containing or lacking the intron, and intron-containing genes must therefore be functional. Recently, evidence from R-loop hybridisation has been used to show that such intervening sequences are indeed transcribed in the closely related species *Tetrahymena thermophila*[7,10]. Thus, the DNA sequences at the boundaries of the intervening element correspond to points at which the precursor rRNA must be spliced.

To study the intervening sequence in *T. pigmentosa* rDNA in more detail, the recombinant plasmids pTp6001 and pTp8002 were constructed from the vector pBR322, as described previously[20]. Briefly, pTp6001 contains a 1.6-kilobase *Hin*dIII fragment from the rDNA of strain 6UM, which includes a part of the 25S rDNA and the intron that interrupts it. Plasmid pTp8002 contains a 1.2-kilobase *Hin*dIII fragment that includes only the corresponding 25S rDNA of strain 8ALP, and lacks the intron. Restriction fragments from these plasmids were sequenced by the chemical cleavage method of Maxam and Gilbert[22]. Position and orientation of the 5′-labelled strands used are indicated in Fig. 1. The entire sequence of the 407-base pair intron and the surrounding rDNA is depicted in Fig. 2, which is a composite of the sequence data obtained from both pTp6001 and pTp8002. In those regions where both DNAs have been analysed (see Fig. 1), the nucleotide sequences are identical.

By directly comparing the sequence from pTp6001 with that from pTp8002, a distinct position is found at which the intron interrupts the coding region (Fig. 3). This is in contrast to all other intron–exon boundaries sequenced to date, in which the exact point of interruption is ambiguous due to the presence of directly repeated nucleotides at the junctions[12,13]. In precursor RNA from protein-coding genes containing introns, the position of the splice within the repeats has been proposed to occur such that all introns begin with 5′-GU and end with AG-3′ (ref. 13). The ends of the *T. pigmentosa* rDNA intron do not obey this rule, and furthermore, are completely different from recent model sequences proposed for mRNA-type intron–exon junctions[1,13,24]. In addition, these rRNA splice points bear no resemblance to the sequences from yeast tRNA intron boundaries[14,15]. At the *T. pigmentosa* rDNA intron junctions themselves, we note the presence of the sequence TCX|XAA at both the 5′ and 3′ ends; whether or not this has a role in defining the splice point in the transcript is unknown.

For recognition and accurate processing of an intron by a splicing mechanism, more information is necessary than is found in the short regions of nucleotide homology seen at mRNA intron boundaries. It is therefore likely that secondary structure of intron-containing precursor RNAs is also important for recognition of the regions where splicing is to occur. In this context, it is interesting that the *T. pigmentosa* rDNA intron itself is fairly rich in potential secondary structure, as determined by computer analysis and indicated in Fig. 2. We also note that both ends of the intervening sequence are rich in AT.

Recently, Allet and Rochaix[23] have determined the sequence of the boundaries of the intron in the *Chlamydomonas* chloroplast 23S ribosomal RNA gene. In this case, too, no similarity to either the mRNA or the tRNA intron junctions of nuclear genes is present. As we have found in *T. pigmentosa* rDNA, the ends of the *Chlamydomonas* rDNA intron are unequivocally defined. Although some sequence homology between these two rDNA introns can be found, any speculations on a model sequence for rRNA intron–exon junctions must await the analysis and comparison of other intron-containing nuclear and organellar rDNAs. If, however, the differences in the splice point sequences of tRNA and mRNA introns reflect a difference in

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* Permanent address: Department of Biology, Yale University, New Haven, Connecticut 06520.

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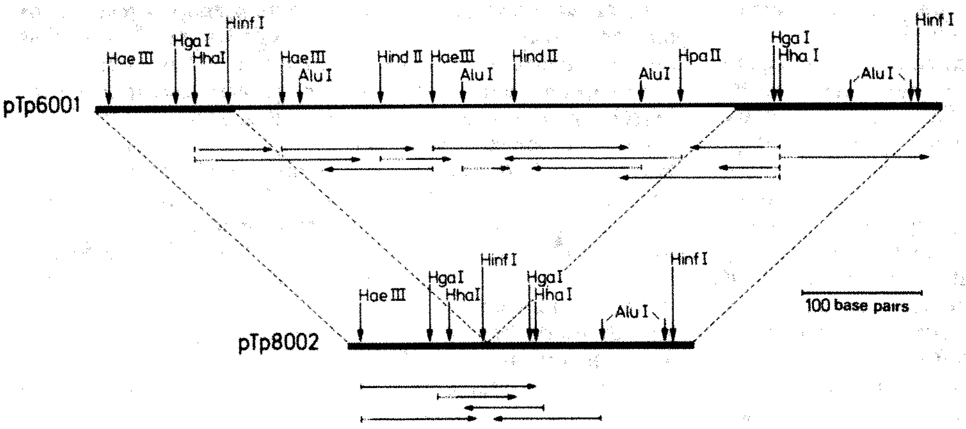

Fig. 1 Partial maps of clone pTp6001, which contains a rDNA intron, and clone pTp8002, which lacks this intron. The connecting lines show the regions of homology between these two clones. All restriction sites are given for those enzymes which were used to prepare fragments for sequencing. Horizontal arrows below each map indicate the position, direction and length of sequence determined for each fragment. After 5′-end-labelling with [γ-^{32}P]ATP and T4 polynucleotide kinase, fragments were strand separated in 0.1 M KOH and 1 mM EDTA, or were digested with a second restriction enzyme, and were then subjected to electrophoresis on 6% or 10% acrylamide gels. DNA sequencing was carried out using the chemical cleavage technique of Maxam and Gilbert[22] with minor modification. The degraded strands were separated on 10%, 20% or 24% acrylamide, 7 M urea gels (2.5 × 200 × 400 mm). After autoradiography, the nucleotide sequences derived from each fragment were stored by computer and analysed for overlaps with each other.

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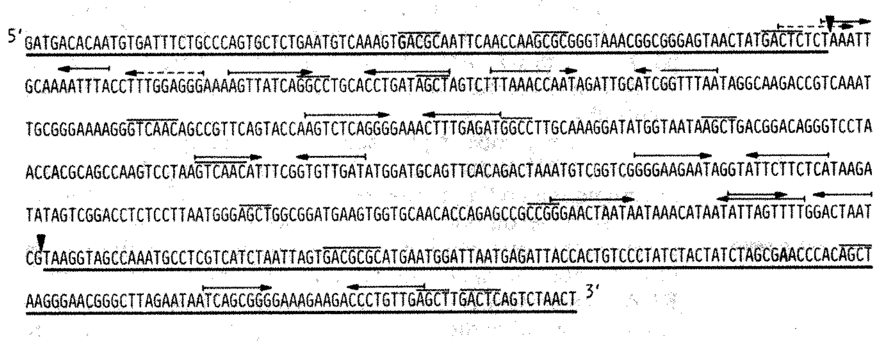

5′ GATGACACAATGTGATTTCTGCCCAGTGCTCTGAATGTCAAAGTGACGCAATTCAACCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTAAATT
GCAAAATTTACCTTTGGAGGGAAAAGTTATCAGGCCTGCACCTGATAGCTAGTCTTTAAACCAATAGATTGCATCGGTTTAATAGGCAAGACCGTCAAAT
TGCGGGAAAAGGGTCAACAGCCGTTCAGTACCAAGTCTCAGGGGAAACTTTGAGATGGCCTTGCAAAGGATATGGTAATAAGCTGACGGACAGGGTCCTA
ACCACGCAGCCAAGTCCTAAGTCAACATTTCGGTGTTGATATGGATGCAGTTCACAGACTAAATGTCGGTCGGGGAAGAATAGGTATTCTTCTCATAAGA
TATAGTCGGACCTCTCCTTAATGGGAGCTGGCGGATGAAGTGGTGCAACACCAGAGCCGCCGGGAACTAATAATAAACATAATATTAGTTTTGGACTAAT
CGTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCATGAATGGATTAATGAGATTACCACTGTCCCTATCTACTATCTAGCGAACCCACAGCT
AAGGGAACGGGCTTAGAATAATCAGCGGGGAAAGAAGACCCTGTTGAGCTTGACTCAGTCTAACT 3′

Fig. 2 Sequence of the 407-base pair rDNA intron and adjacent regions. The sequence presented is a composite of both clones pTp6001 and pTp8002. Only the strand which corresponds to the ribosomal RNA is shown. The intron–exon transition points are indicated by arrowheads; exon regions are underlined. About 100 base pairs of the exon regions were analysed for both clones and were found to be identical. The recognition sites of restriction endonucleases used in the sequence analysis are overlined: AGCT (*Alu*I), GGCC (*Hae*III), GACGC (*Hga*I), GCGC (*Hha*I), GTCAAC (*Hind*II), GACTC (*Hinf*I), CCGG (*Hpa*II). The sequence was screened for potential secondary structure by a computer search for inverted repeat sequences. Only those inverted repeats which are less than 15 nucleotides apart from each other, such that they might form hairpins, are indicated by head-to-head arrows. The inclusion of G·T base pairs, in addition to G·C and A·T base pairs, has been allowed in the inverted repeats. Computer programs used were developed by G. Osterburg and R.S. (unpublished).

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their splicing mechanisms, we suggest that at least one more splicing activity exists which is necessary for the processing of intron-containing ribosomal RNA precursors.

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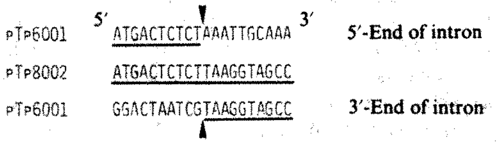

pTp6001 5′ ATGACTCTCTAAATTGCAAA 3′ 5′-End of intron
pTp8002 ATGACTCTCTTAAGGTAGCC
pTp6001 GGACTAATCGTAAGGTAGCC 3′-End of intron

Fig. 3 Sequence at the 5′ and 3′ intron–exon junctions in clone pTp6001 compared with the uninterrupted sequence of clone pTp8002. The unequivocal transition points between intron and exon are indicated by arrowheads. Exons are underlined.

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MATTERS ARISING

Problems with mesospheric electric field measurements

HALE AND CROSKEY[1] have recently reported some unusual results regarding the mesospheric fair weather electric field. These results are extremely interesting for two reasons: the reported values of the electric field are much larger at all altitudes than those previously reported, and the field is observed to increase dramatically at $\sim$65 km altitude. There are several reasons why we feel that the reported data should not be interpreted in the straightforward way proposed by Hale and Croskey, but the possibility that their result is correct certainly justifies considerable further work in this area.

We have performed similar experiments, two of which have been reported[2]. We drew two conclusions from our results. First, the experiment is an extremely difficult one, with large anomalous charging events of both sign seriously and frequently perturbing the potential of the central payload with respect to the medium as determined by the common mode variation of several $\gtrsim 1$ m long antennas. Comparison of these results with unpublished night-time results suggests that the photoelectric effect is not responsible for the observed potentials.

On the basis of body-mounted Gerdien condenser data, Hale and Croskey assert that the potential of their payload did not vary significantly during flight. However, a Gerdien condenser only measures the potential of the payload with respect to the gas passing through the condenser. Essentially all measurements that have been made with extended antennas of the potential of moving vehicles in the 20–80 km altitude range show large, rapidly varying potentials (ref. 2 and personal communications from F. S. Mozer, R. H. Holzworth, and L. C. Hale). On the other hand, Gerdien condenser payload potential measurements seem to show this sort of effect relatively seldom[1,3]. These contrasting results strongly suggest that the potentials seen by the long antenna measurements are being developed sufficiently far from the payload to fool the Gerdien condenser. Gas or ion entrainment effects inside the Mach cone may also be confusing the issue. The fact that large detached potential drops[4–8] and ion entrainment effects[9] have been observed in other admittedly disparate experimental circumstances suggests that similar effects are possible in this case. Regardless of the actual mechanism responsible, a rigorous, physically detailed explanation of the discrepancy between probe and condenser measurements of payload potentials in the mesosphere must be presented by Hale and Croskey (or others) before their measurements can be believed. Cryptic allusions to accumulated experimental experience are no substitute for sound physical argument.

Second, we detected an electric field of 20 mV m^{-1} at an altitude of $\sim$70 km in fair weather, during magnetic quiet, at White Sands, New Mexico, a low latitude site. This measurement was made during a period when the central payload was not anomalously charged. Our experiment and the Hale and Croskey experiment used identical input amplifiers, and were very similar in geometry. Taken together, the measurements imply that Hale's hypothetical global mesospheric circuit must, in fact, be confined to high latitudes or the nightside, or both. This conclusion, and Hale's conclusion that the mesospheric circuit has 'holes' opened in it by auroral activity, has serious observable consequences. The horizontal fringing fields produced at the edges of the 'holes' and the southern edge or the terminator edge of the circuit would be observable at balloon altitudes. Hale *et al.*[3] suggested that the critical observations have been suppressed because balloon observers tend to attribute all data taken during periods of inverted vertical fields to electrified clouds. However, for the case reported by Hale and Croskey, these horizontal fields would not be accompanied by an inversion of the vertical field. Hundreds of hours of balloon data have been taken under circumstances where the fringing fields should have been seen, and they have not been observed (personal communications from F. S. Mozer, and R. H. Holzworth)[3,4]. More important, good agreement is regularly reported between balloon and ionospheric measurements of the horizontal electric field[10,11]. This agreement argues strongly against the existence of these fringing fields, and hence of the hypothetical mesospheric circuit.

A final criticism of both the Hale and Croskey results and the Bragin *et al.*[12] results concerns vehicle dynamics. The large field peaks reported by both groups occur when the payload is travelling at a velocity greater than or equal to Mach 1, and is passing through the region of maximum aerodymamic deceleration. In this context, anomalous 'field values' are not remarkable. What is remarkable is that there are any non-anomalous results from this region.

We would like to stress that there are good physical reasons for knowing the mesospheric electric field[1,2] and the properties of the mesosphere. The points of controversy raised here cannot be resolved without extensive further work, involving parallel approaches, and experiments with controls and comparisons.

E. A. BERING III
J. R. BENBROOK
W. R. SHELDON

Physics Department,
University of Houston,
Houston, Texas 77004

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HALE REPLIES—Although Bering, Benbrook, and Sheldon do not only respond to our letter but also to statements made elsewhere, they have, nevertheless, raised questions that need to be resolved before problems of electrical coupling through the atmosphere can be solved.

We described a quiet-to-disturbed enhancement in the lower atmosphere vertical electric field accompanied by a decrease in a large mesospheric field. The idea of a 'global' mesospheric circuit, possibly related to an aerosol layer, was one suggestion made to explain this effect and the USSR data[1].

We have performed additional measurements which show other aurorally related effects, including field reversals (suggestive of earlier observations by Freier[2] and by Olson[3]) and large amplitude ($\sim$100%) wave-like structure in the lower atmosphere field (L.C.H., C. L. Croskey, and D. F. Young, in preparation). These effects cannot obviously be explained by 'classical' theory, by 'mapping' of magnetospheric fields, or by a simple constant altitude 'global' mesospheric circuit. A mid-latitude sunrise measurement (16 September 1979, 05.30–07.18 LT, Wallops Island, Virginia) showed a relatively low and featureless electric field below 65 km, increasing to about 1.5 V m^{-1} downwards at 20 km, the value measured by Bering *et al.*[4]. The obvious absence of 'anomalous charging' effects on this measurement, where the vehicle potential was determined by a simple 'blunt' probe'[5] in the front surface, gives us confidence that our high latitude results are real, as the vehicle velocity profiles were similar.

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Thin linear electrodes parallel to the flow would seem to be superior to plates or spheres for electric field measurements when dominated by a continuum flow. A wire or rod of length L can be shown to have about the same conductance to the medium as a sphere of diameter L but will have far less photo-emission or turbulent wake effects. Plate electrodes would be sensitive to angle-of-attack variations.

Only a small fraction of the "hundreds of hours of balloon data" cited by Bering *et al.* were accompanied by simultaneous measurements of the auroral electric field, and when they were the agreement was not always good even during fair weather[6]. The limited extent of 'fringing fields' of even a non-global mesospheric circuit would be such that I doubt whether there is sufficient data to reject such a possibility out-of-hand, although this is not necessarily the only coupling mechanism. The local effects of aurorally produced charge separation, suggested by Freier[2], would apparently be capable of explaining some of the observations, particularly the reversals.

I echo Bering *et al.*'s plea for the acquisition of more data 'with controls and comparisons'. Perhaps this can be done by the international community during the forthcoming Middle Atmosphere Program (MAP). The several hundred meteorological class rockets necessary for a reasonable global description of the electric field in a variety of geophysical conditions is a minute effort compared to the tens of thousands of rockets that have been launched to acquire mesospheric wind and temperature data. These measurements should certainly be accompanied by balloon measurements but note that our coupling effect would not have been detected at intermediate altitudes.

LESLIE C. HALE

Ionosphere Research Laboratory,
The Pennsylvania State University,
University Park,
Pennsylvania 16802

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GABAergic mechanisms in the substantia nigra

MARTIN AND HAUBRICH[1] have confirmed the original reports[2,3] of contralateral rotational behaviour following unilateral injections of the γ-aminobutyric acid (GABA) agonist muscimol into the zona reticulata of the rat substantia nigra. They have also confirmed a previous report[4] that intranigral perfusion of muscimol increases ^{3}H-dopamine (DA) efflux in striatal perfusates loaded with radiolabelled DA precursor. We believe their conclusion, that contraversive rotation to intranigral muscimol is mediated via activation of nigrostriatal DA neurones, to be erroneous.

Unilateral intranigral injections of muscimol ($100\ \text{ng}\ \mu\text{l}^{-1}$ saline) were made[2] into the substantia nigra of unlesioned rats or of rats in which the ipsilateral nigrostriatal DA system had been destroyed 21 days previously by intracerebral injection of 6-hydroxydopamine (6-OHDA $8\ \mu\text{g}$ per $4\ \mu\text{l}$) into the medial forebrain bundle[5]. Rotational behaviour was quantified in automated rotometers[5] for 45 min and animals then taken for neurochemical analyses. 6-OHDA-lesioned rats with 90% destruction of the nigrostriatal DA system, as indexed by striatal tyrosine hydroxylase activity, exhibited vigorous contralateral rotation to intranigral muscimol that was indistinguishable from that shown by nonlesioned rats (Fig. 1). We therefore conclude that these circling responses are independent of dopaminergic mechanisms. Note that there is already evidence that nigral non-dopaminergic neurones control postural asymmetry[6], and that Martin *et al.*[7] were unable to demonstrate consistent antagonism of muscimol-induced turning by haloperidol. We find doses of haloperidol that are clearly blocking striatal DA receptors to have either very weak or no effect on rotational responses after intranigral administration of GABAergic drugs[8].

In unlesioned rats, striata ipsilateral to intranigral injections of 100 ng muscimol showed[8] elevated concentrations of DA (+98%, $P<0.02$) and of the intraneuronal[9] metabolite dihydroxyphenylacetic acid (+71%, $P<0.01$) but not the extraneuronal metabolite[9] homovanillic acid (+4%, NS). This neurochemical profile is consistent with that of the increased synthesis and decreased release of DA which follows inhibition of impulse flow in nigrostriatal DA neurones[10], and therefore with a GABAergic inhibition of activity of nigral neurones[11]. The finding by Martin and Haubrich[1] of elevated ^{3}H-DA in striatal perfusates following intranigral muscimol could in part reflect the elevated synthesis of DA known to occur after inhibition of impulse flow in nigrostriatal DA neurones[10].

Rotation after intranigral muscimol is most enduring and rats taken for neurochemical studies at 45 min after such treatment were rotating vigorously at this time. The resultant striatal neurochemical profile was consistent with reduced dopaminergic activity. In their reply Martin and Haubrich concede that DA release may be reduced at 45 min after intranigral application of muscimol, when rats would still be rotating. This is inconsistent with their proposition that striatal DA release mediates the contralateral rotational response to intranigral muscimol. We have recently shown[12] that destruction of striatal cell bodies and striatal efferents by intrastriatal injections of kainic acid fails to attenuate turning following intranigral muscimol. Therefore striatal DA release cannot mediate this response.

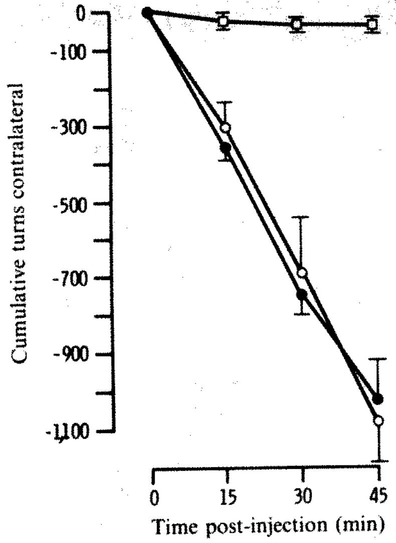

Fig. 1 Rotational responses to unilateral intranigral injection of 100 ng muscimol in male Sprague–Dawley rats with (○, $n = 5$) and without (●, $n = 10$) destruction of the ipsilateral nigrostriatal DA pathway induced with 6-OHDA. Intranigral injections were made under light ether anaesthesia as previously described[2], in $1\ \mu\text{l}$ saline. 6-OHDA ($8\ \mu\text{g}$ per $4\ \mu\text{l}$ saline ascorbate) was injected 21 days previously into the medial forebrain bundle[5]. Control animals received $1\ \mu\text{l}$ saline into the nigra after 6-OHDA lesions (□, $n = 5$); such injections made into the nigra of unlesioned animals also induced negligible circling[2]. Rotational behaviour was quantified in automatic rotometers[5].

Thus our behavioural findings suggest that contralateral rotation following intranigral injection of GABAergic drugs is not due to activation of the nigrostriatal dopamine system and our neurochemical findings suggest that the activity of this system can be inhibited by this procedure[13].

JOHN L. WADDINGTON

Division of Psychiatry,
MRC Clinical Research Centre,
Watford Road,
Harrow, Middlesex, UK

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MARTIN AND HAUBRICH REPLY—We have reported that the efflux of ^{3}H-dopamine (DA) from the ipsilateral striatum increases during contraversive turning evoked by the intranigral infusion of muscimol in the rat. From these data we suggested that the release of striatal DA may mediate the muscimol-evoked contralateral turning[1]. In a separate study using immobilised cats, Cheramy and coworkers have also demonstrated that the intranigral infusion of muscimol elicits a similar efflux of ^{3}H-DA from the ipsilateral striatum[2,3]. The results from both studies indicate that dopamine release occurs in the ipsilateral striatum during the intranigral infusion of muscimol. We feel that our interpretation of the push–pull data are correct.

Waddington has challenged our suggestion that the release of DA might mediate muscimol-induced turning. This finding is based on two of his observations. First, the destruction of 90% of the striatal DA neurones with microinjection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle did not attenuate the turning evoked by the application of muscimol into the substantia nigra. Although the seeming complete lack of diminution in muscimol-evoked turning in 6-OHDA-treated animals is somewhat surprising, it does not preclude the possibility that the remaining 10% of the DA neurones might mediate the turning, especially in view of the supersensitivity which develops in postsynaptic DA receptors after 6-OHDA treatment[4]. Therefore, this negative finding does not incontrovertibly exclude the possibility that muscimol causes turning by stimulating DA release. Waddington has apparently not determined whether or not DA release occurs in the striatum of the 6-OHDA-lesioned rats following the intranigral microinjection of muscimol, nor whether haloperidol depresses significantly the muscimol-induced turning in 6-OHDA-lesioned rats as in intact animals[5–7], and therefore these data are not conclusive in ruling out the role of dopamine release in the turning activity evoked by intranigrally applied muscimol.

Waddington also suggests that muscimol inhibits the release of DA, whereas we have reported an enhanced DA release. Our conclusion is based on direct measurement of DA release from dopaminergic neurones during the time of the muscimol infusion, whereas those of Waddington are based on indirect evidence obtained from measurement of levels of DA and metabolites in tissue dissected 45 min after the microinjection of muscimol. We feel, therefore, that there is no basis for comparing the two dissimilar pieces of information and that to debate the point is meaningless. We feel strongly that we are measuring DA release.

As haloperidol only partially antagonised the muscimol-evoked turning in another study[5], we postulated the possible activation of at least one other neuronal system, perhaps GABA-like, in the activation of the muscimol-evoked turning. We did not wish to imply that DA release is the sole mediator of the turning. Contrary to Waddington's assertion, however, we feel that our evidence is quite compelling in indicating that striatal DA release, at least in part, may mediate the turning induced by intranigrally applied muscimol.

G. E. MARTIN
D. R. HAUBRICH
Merck, Sharp and Dohme,
Research Laboratories,
West Point, Pennsylvania 19486

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Is corticotropin releasing factor modulated vasopressin?

A RECENT report by Gillies and Lowry concludes that corticotropin releasing factor (CRF) is arginine vasopressin (AVP) modulated by other hypothalamic factors[1]. This claim is based on several experimental observations. First, fractionation of a crude extract of stalk median eminence (SME) showed that three factors were required for full biological activity. Second, on Biogel P2, the major peak of CRF activity eluted in the expected position for AVP. Third, removal of AVP by cross-reacting the SME extract with vasopressin antisera dramatically reduced the CRF activity. Finally, SME extract from Brattleboro rats, which lack AVP genetically, contains 20% of normal CRF activity.

Our laboratory has also published evidence that the CRF activity of SME extract contains at least two factors which are required for full CRF activity[2]. Furthermore, on the basis of studies of the CRF-like activity of peptides related to oxytocin and vasopressin, we showed that the greatest CRF-like activity was displayed by AVP, in which 40 ng contained 27% of the CRF-activity of one median eminence[3]. However, because we observed normal CRF-like activity in a SME extract of DI-HOMO (Brattleboro) rats, we discarded the notion that AVP is the physiological CRF. On the basis of this evidence, we have proposed previously that the structure of CRF may closely resemble that of AVP.

Because our data comparing the CRF-like activity of Brattleboro SME extract with that of normal rates have not yet been published, we repeated these experiments in our laboratory. We used a semi-automated *in vitro* bioassay for CRF in which the ACTH released from normal rat pituitary halves is determined by measuring the corticosterone secreted by normal rat adrenal quarters in an automated superfusion system[3]. This bioassay has a linear dose–response relationship at 0.05–2.0 median eminence equivalents.

Median eminence extract from normal Long–Evans, normal Sprague–Dawley and DI-HOMO Brattleboro rats was prepared in 0.1 M HCl at a concentration of 25 µl per SME[4]. The test dose in all experiments was 20 µl, 0.8 SME equivalents, which was well within the linear portion of the dose–response curve for this bioassay[3]. After a 2-h preincubation, one set of each pair of pituitaries was incubated for 1 h with 20 µl of SME extract from Sprague–Dawley rats; the second set of each pair of pituitaries was incubated in buffer alone to serve as a control. After a second preincubation of 1 h, the test sample was incubated with one set of each pair of pituitaries; again, the other set served as a control. ACTH released by the pituitary halves, as well as any ACTH-like activity in the SME extract, was measured in a continuous flow, automated, *in vitro* superfusion apparatus containing adrenal quarters. This assay is described in detail in ref. 3.

Stalk ME extract of Brattleboro rats ($n = 7$) contains 98% (±s.e., 12) of the CRF-like activity of normal Sprague–Dawley rats ($n = 6$) and 96% (±s.e., 12) of that of normal Long–Evans rats ($n = 4$). Therefore, we conclude that Brattleboro rats, which lack AVP and its associated neurophysin[5–7], contain essentially normal amounts of hypothalamic CRF activity, and, presumably, normal amounts of CRF. The only other published observation which shows a dramatic reduction (~60%) of CRF-like activity in SME extract from Brattleboro rats uses a dispersed cell pituitary assay system[8], as do Gillies and Lowry. All workers seem to agree, however, that the Brattleboro rat can respond to stress with the secretion of near-normal levels of corticosterone[8–10]. Watkins observed a positive immunoperoxidase staining reaction in the paraventricular and supraoptic nuclei of Brattleboro rats when anti-[8-lysine]-vasopressin serum was used[11]. Lutz-Bucher *et al.* have concluded that the anterior pituitary contains receptor sites for vasopressin which are distinct from those for hypothalamic CRF[12]. In this context, it is interesting that Zimmerman has reported immunological evidence for another peptide in Brattleboro rats which cross-reacts weakly with anti-vasopressin[13] and may be related to the physiological CRF.

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A. FRANCES PEARLMUTTER
LINDA DOKAS
ANN KONG
RALPH MILLER*
MURRAY SAFFRAN
Medical College of Ohio,
Department of Biochemistry,
Toledo, Ohio 43699
**University of Kentucky Medical School,*
Department of Pharmacology,
Lexington, Kentucky

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GILLIES AND LOWRY REPLY—The above response to our paper[1] describes an experiment in which no difference was observed between the CRF activity of Brattleboro hypothalmic extracts and that of normal rats. As Brattleboro rats are deficient in vasopressin (VP), the authors conclude that VP is not CRF. However, our recent work with extracts of stalk-median eminence (SME) from Brattleboro rats[2] has added support to our idea that CRF is modulated VP. We found that Brattleboro SME contained approximately one fifth the CRF activity found in normal SME. Similar observations have been made using both isolated pituitary cells[3] and pituitary quarters[4].

Gel filtration of Brattleboro SME revealed only two small discrete peaks of CRF bioactivity corresponding in position and size to the Vo and Ns peaks found after chromatography of normal rat SME[1]. The third and major CRF peak found in normal rat SME, which coelutes with synthetic AVP and resembles AVP in our immunoassay and CRF bioassay, was absent from the Brattleboro chromatogram. Furthermore, addition of synthetic AVP in amounts equivalent to that found in normal SME (10 ng AVP per SME[5]) to extracts of Brattleboro SME completely restored the CRF activity to that of normals[2]. This observation has now been confirmed in a different assay system[4]. These results coupled with the facts that (1) we have consistently been able to combine the Vo and Ns CRF peaks from the chromatogram of normal SME with either the major AVP-CRF peak or equivalent amounts of synthetic AVP to restore full CRF bioactivity[1] and (2) no analogue of VP has been found with CRF bioactivity greater than AVP[5,6], suggest to us that we have ample support for our conclusion that the AVP-CRF peak is true vasopressin, and not merely related in structure to it.

The above response cites Zimmerman's preliminary observation on the possibility of a vasopressin-like peptide in the Brattleboro rat which may be related to CRF. In a subsequent report[7], however, it was stated that homozygous Brattleboro rats lack VP and its associated neurophysin and the occasional staining of some cells in the SON and PVN of these rats with their antiserum to AVP "appears to be due to slight reactivity of this antiserum to oxytocin". It should also be noted that the antiserum used by Watkins[8] had 14% cross-reactivity with oxytocin and (8-arginine)-VP, so one cannot exclude the possibility of a positive immunohistochemical reaction in the SON and PVN of homozygous Brattleboro rats being due to oxytocin.

The immunocytochemical studies of Stillman *et al.*[9], showed that VP and its associated neurophysin are stimulated by adrenalectomy, and their content in pathways to the portal capillaries is regulated by glucocorticoids, suggesting an important role for VP in corticotropin release.

As Pearlmutter *et al.* state above, all agree that Brattleboro rats are able to respond to stress. However, results of *in vivo* experiments are contradictory as to whether this stress response, as measured by adrenal activity, is normal[10] or significantly subnormal[11-13] and, if an abnormality exists, whether it is at the hypothalamic, pituitary or adrenal level. We feel that many of the contradictions from *in vivo* experiments could be clarified, and a truer measure of CRF release in response to stress would be made, if ACTH release were the parameter measured, because supramaximal stress stimuli may lead to the release of CRF (the non-VP components in Brattleboro rats) and consequently ACTH, in amounts exceeding those necessary for maximal stimulation of the adrenal glands. This could explain why Yates *et al.*[12] observed that a deficiency in the stress response of homozygous Brattleboro rats was evident only when submaximal stresses were investigated.

We also suggest that the bioassay system used in *in vitro* experiments contributes to this controversy on the role of VP as a CRF. First, isolated cell systems in general, and especially the perfused isolated cell system, as bioassays for CRF are far more sensitive than those using pituitary halves or quarters [we were able to demonstrate a threefold potentiation of 0.025 Brattleboro SME per ml by 0.25 ng AVP per ml (ref. 2)]. This must account for the fact that we were able to observe discrete peaks of CRF bioactivity in the chromatogram of extracts of small numbers of rat SME, and identify one peak as VP[1]. Second, fresh excised rat anterior pituitaries contain significant amounts of VP[15] which disappear from the tissue only after incubation for 3 h, suggesting specific binding to receptors. Therefore, in bioassays which use freshly removed tissue or incubated tissue which has been previously stimulated with solutions containing VP, such as a hypothalamic extract (as in the bioassay system used by Pearlmutter *et al.*[6]), it is possible that no difference would be found between the CRF activity of Brattleboro and normal rat SME, as the VP component of CRF would already be in the tissue. It is interesting to note here that Buckingham and Leach[4], who use a CRF bioassay involving rat anterior pituitary quarters coupled with the cytochemical bioassay for ACTH[16], have reported diminished CRF bioactivity in Brattleboro SME. As well as the difference in the ACTH bioassays used, the discrepancy with the findings of Pearlmutter *et al.* may be due to the duration of static incubation of extracts with anterior pituitary tissue—15 min for Buckingham and Hodges[16] and 60 min for Pearlmutter *et al.*[6], compared with 2–3 min contact of stimuli with isolated cells in our perfused system. The net CRF bioactivity observed could depend on the breakdown of both CRF and ACTH in the incubation medium.

G. E. GILLIES
P. J. LOWRY
Pituitary Hormone Laboratory,
St Bartholomew's Hospital,
51–53 Bartholomew Close,
London EC1, UK

1. Gillies, G. & Lowry, P. J. *Nature* **278,** 463–464 (1979).
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BOOK REVIEWS

What the tortoise never said to Achilles*

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CALCUTTA

Oliver Penrose

Gödel, Escher and Bach: An Eternal Golden Braid — A Metaphorical Fugue on minds and machines in the spirit of Lewis Carroll. By D. R. Hofstadter. Pp.777. (Basic Books: New York, 1978; Harvester: Brighton, UK, 1979.) $18.50; £10.50.

ACHILLES had overtaken the Tortoise, and had seated himself comfortably on its back. "So you've got to the end of our race — er, our book" said the Tortoise. "Even though it DOES consist of an infinite variety of self-referring statements, curious puns, paradoxes, and speculations? I thought some wiseacre or other had proved that the thing couldn't be done?"

"It can be done", said Achilles. "It HAS been done! *Solvitur legendo*. You see each pun or paradox only took HALF as long to read as the one before; and so . . ."

"But the Index", interrupted the Tortoise, "how did you finish THAT? Every time you came to the entry 'Achilles: mapped on to ant colony 318, 324' you surely had to turn back to the middle of the book, so how could you EVER finish?"

"That splendid index", murmured Achilles. "It's true I did have some trouble getting past the entry 'Zen Buddhism; computers and, 625 — 26' especially as one of the chapters is based on Zen kōans, but even so I'm sure I'd have beaten you if I hadn't spent so long trying to do that fascinating MIU puzzle in chapter 1".

"Oh yes", recalled the Tortoise, "given the string of letters MI, and the rules for producing new strings:

1. If your string ends with I, you can add a U at the end.
2. If your string is Mx, where x is any string, you can change it to Mxx.
3. If your string contains the sequence III, you can replace that sequence by U.
4. If your string contains the sequence UU, you can delete that sequence.

can you produce the string MU?"

"Well, I'm only a simple warrior", said Achilles "and I never did manage to produce MU, but I thought the puzzle was a BRILLIANT way of introducing the idea of a formal logical system."

"And formal logical systems are very important", said the Tortoise, "because the centrepiece of the book is a sketch of the proof of Gödel's theorem: that practically any formal logical system contains statements which can't be either proved or disproved within the system."

"Ah yes, that proof of Gödel's theorem", sighed Achilles. "Very entertaining, like the rest of the book, but even after reading it an INFINITE number of times I still don't think I understand it."

"Each reading taking only half as long as the previous one, of course", mused the Tortoise. "But I think the author's intention was not so much to give a crystal-clear explanation of Gödel's proof (he might possibly have done that better by making more use of Cantor's diagonal construction) as to exploit the idea of self-reference to the full. So he gives, as an analogue of Gödel's undecidable formal proposition, the following English statement: 'yields falsehood when preceded by its quotation' yields falsehood when preceded by its quotation."

"That must be the grandmother of all confusing self-referring sentences", said Achilles, mopping his brow. "I never DID manage to decide whether that one is true or not. But rather than get worried about it I turned to the Escher lithographs which he uses so delightfully to illustrate many of his ideas — for example, the picture of someone in an art gallery looking at a picture of a town which contains an art gallery which contains . . .".

"Do you mind", interrupted the Tortoise, "you are sticking your sword into my foot."

"MIND?", said Achilles, waving the sword excitedly. "That's just the kind of thing the rest of the book is about. What is a mind? Where is meaning located? How is knowledge represented? Can a computer be conscious? Can animals with very primitive nervous systems be said to think? . . ."

"More easily than featherbrained warriors", muttered the Tortoise testily.

"Do you remember the description in the book of Terry Winograd's artificial intelligence device SHRDLU," went on Achilles, as if the Tortoise had not spoken, "which can move solid objects around in response to commands like 'find a block which is taller than the one you are holding and put it into the box'?"

"With comments by 'the newly developed human being, Dr Tony Earrwig' who seems to be related in a mixed-up way to SHRDLU's creator" added the Tortoise. "By the way, if you had to read the chapter about Gödel's theorem so often, I'm amazed that you finished the book so quickly."

"Well, I have to admit", said Achilles, "that to help me catch up with you, I only read every other chapter. The book still seemed to make sense though."

"What?" The Tortoise was horrified. "You missed the best part. Those chapters are all about US. And you missed my wonderful invention, the Tortoise property, enjoyed by every number that is the difference of two primes".

"Are you sure you don't mean the Goldbach property, which is that the number is the SUM of two primes?"

"No, the difference. The Goldbach property is VERY dull: you can always tell in a finite number of steps whether a given number has it or not, whereas if a number does not have the Tortoise property you could go on trying primes for ever. Besides that", continued the Tortoise, "you missed some extremely ingenious dialogues, which are verbal imitations of contrapuntal devices used by Bach in his much-loved works such as the Musical Offering. They provide welcome points of relaxation between the more serious chapters that you did read."

"With Bach as a model, the book must be even better constructed than I thought", said Achilles. "And the author's enthusiasm certainly carries you along as you read. But don't I see our mixed-up relatives Les Cahil and Sir Otto E. over there? Let's go and join them". ☐

Oliver Penrose is a Professor of Mathematics at the Open University, Milton Keynes, UK.

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*With apologies to Lewis Carroll "What the Tortoise said to Achilles", *Mind*, n.s.4 (1895), pp.278-80, reprinted in pp.43-45 of the book reviewed here.

Interferometric spectroscopy

E. R. Pike

The Principles of Interferometric Spectroscopy. By John Chamberlain. Pp. 347. (Wiley: Chichester, UK, 1979) £20.

THIS book is the result of the completion, collection and revision (to include up-to-date advances, by Chantry and Stone) of material left at the time of John Chamberlain's death in 1974. It represents an attempt to bring together the whole field of interferometric spectroscopy at a practical working level in a single self-contained and readily accessible fashion.

As a knowledgeable and able practitioner Chamberlain leads authoritatively from the work of Michelson through a historical introductory chapter to an exposition of introductory theory in chapters 2, 3 and 4. These three chapters cover the ground of the Fourier transform, sampling theory and related topics, the mathematical description of the light field, including the concepts of the analytic signal, coherence and the coherency matrix, and the theory of the practical interference function. The fast Fourier transform is treated in the book's final chapter. Much of this didactic material is, of course, readily available on the bookshelf but as presented here forms a particularly thorough and logical operational approach. A few purist quibbles can be raised here and there as, for example, on page 73 where it is stated that "Physically, the electric and magnetic wave-fields are real quantities . . .". Are they? In this book the only signals implicitly used are photon annihilation rates at the detector. If we were to measure an electron detection rate, say, in another branch of physics, would we assert that they derived from a real Schrödinger wave?

The theory of transform spectroscopy forms the core of the text in chapters 5, 6 and 7. Here are treated, respectively, the measurement of spectral line-shapes from the visibility curves, then non-dispersive and finally dispersive spectroscopy. Again, as in the previous section, the work is notable for its thoroughness and logical development. Full details are given of the effects and practical methods of dealing with them of truncation, sampling and phase errors. Apodisation schemes, for example, are reviewed in considerable detail.

A general information-theoretic approach is absent. The Fourier transform is taken for granted to be the magic requirement to derive what went in from what came out. The method of interferometric spectroscopy, however, is an ill-conditioned first-order Fredholm problem and thus insoluble without a 'model' for the inversion to inject sufficient extra *a priori* information to remove the ill-conditioning. The Fourier transform, viewed in this light, performs a least-squares fit to a model spectrum consisting of a truncated sum of cosine waves. Although this provides an extremely practical approach to retrieving such information about the spectrum as is transmitted by the instrument it surely does not squeeze out the last drop, and a discussion on these more general lines would have been interesting.

A competent chapter 8 is devoted to various experimental considerations such as finite apertures, imperfect mirrors, modulation schemes and rapid scanning. Perhaps in experimental matters the rival book of R.J. Bell (*Introductory Fourier Transform Spectroscopy*, Academic Press, 1972) is more extensive.

The importance of interferometric spectroscopy derives in large part from the so-called Fellgett advantage, and it was a disappointment to the reviewer to find, yet again, both in the introductory chapter and in chapter 9 on noise, the commonly propagated fallacious explanation that the improvement is due to seeing all components of the spectrum at once. To a mathematician the nature of a function does not change in its Sturm-Liouville transform and one would naturally ask why the grating is not superior because it sees all delay times at once! This (in his own words) "difficulty in navigating the trickier fine points" shipwrecks him on the erroneous conclusion (page 263) that a multiplex advantage does not exist in the photon noise-limited region, although, apparently without its relevance fully sinking in, the result of Kahn (correct within a factor of 2) is given on page 302. He founders also on page 299, getting completely wrong the intensity and photoelectron fluctuations for quasi-monochromatic light. "Gaussian" and "Bose-Einstein" should be replaced by "negative-exponential" and "geometric" in this discussion.

Apart from these blemishes, the first of which seems to be indigenous to the subject, the book can be recommended to anyone entering this field, even at the specialist price of £20. ☐

E.R. Pike is Deputy-Chief Scientific Officer in the Physics Group of the Royal Signals and Radar Establishment, Great Malvern, UK.

Small mammal ecology

Ian Linn

Ecology of Small Mammals. Edited by D.M. Stoddart. Pp. 386. (Chapman and Hall: London, 1979.) £15.

THIS book is an interesting and worthwhile attempt, in a series of essays by specialist authors, to review the state of the art in small mammal ecology and to indicate the areas in which this field of study still requires more intensive investigation. As a review it succeeds quite well. The individual essays reveal, in their expertise, the undoubted skill of the authors in their special fields, and the general coverage makes the whole exercise useful. Nevertheless, there is a lot of bias in this coverage, and it seems to me that the Editor might have exerted more discipline on his contributors, to ensure that the subject was dealt with more evenly.

Thus, the emphasis of the book is firmly laid on the ecology of the rodents of north temperate regions. This emphasis is a simple reflection of the animals and regions about which most is known, but the Editor on the first page of his preface defines small mammals as those weighing less than 5 kg, and there are plenty of insectivores, carnivores and lagomorphs in that category, some smaller primates and even a few artiodactyls. There are honourable mentions of some of these here and there, and a couple of special chapters devoted to bats and marsupials, but more is known than would appear from the text. Even in the case of rodents, which are well covered on the whole, the special problems of tropical animals receive less attention than I would have expected.

If one continues to examine the book with comprehensive coverage in mind, some regrettable general lacunae are to be found. Here I would pick out particularly the topic of behavioural ecology. It is a pity that this aspect of the biology of mammals, which throws such considerable light on their place in the ecosystem, is treated so sparsely. Similarly it is a matter for regret that food habits and feeding ecology are only briefly mentioned.

Perhaps it is too much to expect comprehensiveness in a book of this size, and it should simply be seen as a collection of more or less separate essays, reflecting the views of the authors about certain undoubtedly important areas of knowledge. As such the book is valuable, and can be read with profit by any serious ecologist, and by many others (doctors, veterinarians, pest controllers) whose interest in mammal ecology is more marginal. Professor George Varley once remarked to me that it was time the mammal ecologists got their subject to the same level as the entomologists. Perhaps we still have some way to go, but this book is proof that tremendous strides have been made towards this desirable goal. ☐

Ian Linn is Lecturer in the Department of Biological Sciences at the University of Exeter, UK.

Realm of pericyclic reactions

C. W. Bird

Organic Reactions and Orbital Symmetry. By T.L. Gilchrist and R.C. Storr. Second edition. Pp.311. (Cambridge University Press: Cambridge, 1979.) Hardback £25; paperback £8.50.

THE application of the concept of conservation of orbital symmetry in concerted reactions has had a profound effect upon the development of organic chemistry since its independent enunciation in 1965 by the late R. B. Woodward and R. Hoffmann, and H. C. Longuett-Higgins and E. W. Abrahamson. Not only did the concept facilitate the rationalisation of much knowledge, but more importantly it proved to have considerable predictive capability. The first edition of this book in 1972 provided a timely exposition of the rapidly expanding area, following hard upon the heels of the proselytising 'little blue book' of Woodward and Hoffmann with its peremptory claim "Violations. There are none!".

The reader of this most welcome second edition will search in vain for the dénouement usually visited upon such seeming arrogance by Murphy's Law. He will, however, find a lucid account of the theoretical origins and multifarious applications of this concept. A resumé of the chapter headings provides a guide to the topics covered: classification and investigation of reaction mechanisms (20 pages); theory of concerted reactions (23); electrocyclic reactions (26); cycloadditions: introduction (36); cycloadditions and eliminations involving six electrons (57); other cycloadditions (69); and sigmatropic rearrangements and related reactions (63). While providing in a largely non-mathematical form accounts of the various alternative approaches to the underlying theory, stress is now placed upon the particular merits of the frontier molecular orbital approach. However, as might be expected of practising experimental chemists, the authors have emphasised throughout the synthetic applications of these pericyclic reactions. They have also succeeded admirably in their intention of providing a perceptive up-to-date introduction to the subject rather than an exhaustive survey, although extensive references to other books, review articles and original papers are provided for those requiring further information.

Like its predecessor this book provides an excellent introduction to the realm of pericyclic reactions, and can be unhesitatingly recommended to both neophyte and pedagogue. □

C. W. Bird is Reader in the Department of Chemistry at Queen Elizabeth College, University of London, U.K.

Laser speckle

T.S. McKechnie

Laser Speckle and Applications in Optics. By M. Françon. Pp.161. (Academic: New York, London and San Francisco, 1979.) $16.

THIS book gives a concise, well ordered account of the origin and nature of speckle and of how it can be applied usefully. There are many illustrations in the book (158 in total), and with an absence of all but the most trivial mathematics, it makes pleasant and comfortable reading. The book has ten chapters, each broken into about six sections.

Chapter 1 begins by considering the image of a point source, and from this very humble starting point it progresses to deduce the image of many point sources, that is, a speckle pattern. Chapters 2 to 5 consider the effects of changing some of the variables which govern the speckle patterns. In addition to many more, consideration is given to such things as changing the plane of observation of the speckle pattern and changing the orientation and wavelength of the illumination. To one who might regard speckle patterns as being random and ungoverened by sensible laws, the results outlined in these chapters will come as a pleasant surprise. There are many ways in which speckle patterns behave simply and predictably, and the author has brought this out very clearly. The material comprising these chapters forms a basis for the applications which are dealt with in the remaining chapters.

Image processing is considered in chapter 6 and, along with several others, techniques are described for extracting the difference between two images, as are techniques for coding and decoding images. Chapter 7 is concerned with the study of displacement and deformation using speckle patterns. Chapter 8 concerns speckle in astronomy and the elegant method of Labeyrie for gaining diffraction limited performance from the largest telescopes. Chapter 9 deals with the measurement of surface roughness and Chapter 10 deals with various other applications of speckle patterns which do not fall within the previous categories.

The book presents a simple concise account of the subject. Researchers will find it very useful as a book for first reference; and, for those who wish to go deeper into the subject, an extensive list of references is given. □

T. S. McKechnie is in the Department of Mechanical Engineering at Loughborough University, Loughborough, UK.

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Geophysics of Mars

Garry E. Hunt

Geophysics of Mars. Developments in Solar System and Space Science, Vol.4. By R. A. Wells. Pp.678. (Elsevier Scientific: Amsterdam, Oxford and New York, 1979.) $87.75; Dfl. 180.

FOR centuries, the planet Mars has been the centre of intrigue for Mankind. Across the cold icy darkness of space, there has always been speculation that life could have developed on our planetary neighbour. In recent years, detailed observations from spacecraft have added to our measurements of the Martian environment by Earth-based telescopes, so that we are now building a fairly comprehensive picture of the planet now, and some interesting indications of its past. In this monograph, Dr Wells has produced a huge text which tries to put into perspective our current understanding of the geophysics of Mars. I believe he has largely succeeded in a well written and carefully illustrated text.

Naturally, the structure of the text and its content must have some personal biases, but fortunately all of the eight chapters have a wealth of references to supplement the material, so that the reader will not feel that his understanding of Mars is too strongly influenced by the author's own views. We are led into the discussions of Mars through the physical interaction of the atmosphere and surface. The importance of the Martian atmosphere in shaping some of the surface features and the minor constituents in providing information on the past history of the planet are reviewed. The chapter continues by introducing the basic spectroscopic matters related to the detection of atmospheric constituents such as CO_2, CO, O_3 and H_2O, their variability, and the basic techniques of determining the surface pressure and aerosol content of the atmosphere. Mars does possess a very exciting meteorology and I personally think it is unfortunate that this characteristic has not been highlighted more. There is some discussion of the cloud systems seen on the planet, but those seen by Viking in the Tharsis area, such as bore wave clouds, are unfortunately incomplete. The discussion of the dust storm generation misses out the fundamental observations by the Viking imaging and infrared instruments which have clearly shown that separate mechanisms are responsible for the preperihelion and perihelion storms.

Two of the most controversial aspects of Mars are concerned with the obscuration of the dark surface markings at short wavelengths and the progressive, seasonal, cyclic increase in their darkness at long wavelengths. These aspects are more familiarly known as the blue haze and the seasonal wave of darkening, respectively. A complete chapter is devoted to these matters. The discussions are very thorough. The reader will also be introduced to some of the problems and applications of Mie scattering theory in a simple and effective way which is always useful.

The next three chapters deal with different aspects of the basic surface properties of the planet. The geological aspects of Mars are built up from our early telescopic observations and subsequently improved with the spacecraft data through the 1960s until the present Viking data is available. A brief discussion is given of the problem of estimating the age of a surface by crater counting, but I am not sure whether the reader will necessarily know which set of results he should believe at this stage. The remaining two chapters of this sequence cover the Martian topography and the linear features. It may be sad for some readers that Lowell's infamous canals do not relate to any of the Martian features. However, this situation is in itself a valuable calibration, and a direct indication of the care we must take in interpreting visual observations.

Two further chapters cover the internal structure of the planet, mantle convection and the Martian moons, Phobos and Deimos. The monograph concludes with a detailed discussion of the first few months of the Viking view of Mars. As a result, a great deal of the seasonal aspects, and all the annual variations of the atmosphere, are not included.

This book has been written with great care, and there is every indication that the author has devoted himself to this task of placing on record the geophysics of Mars. I certainly believe that it is a valuable addition to the discussion of the Martian environment, and is well supplemented by a large number of high quality diagrams and photographs. But how many people, or indeed libraries, can really afford £41? I wish publishers would try to be a little more realistic in their prices; then good books, such as this one, would reach a much bigger audience. □

Garry E. Hunt is Head of the Laboratory for Planetary Atmospheres, Department of Physics and Astronomy, University College, London, UK.

Electron microscopical techniques

S.J. Kirchanski

Electron Microscopy and Cytochemistry of Plant Cells. By J.L. Hall. Pp.444. (Elsevier/North Holland: Amsterdam and New York, 1979.) $46.25; Dfl.95.

THIS book is indeed a pleasant surprise; all too often edited volumes are very uneven in quality and contain unfocused or inappropriate chapters. On the contrary, in this work all the chapters are of good quality and contribute an overall unified aspect to the volume. There are seven chapters which cover preparation of thin sections, enzyme cytochemistry, labelled antibodies and lectins, ion localization, autoradiography, freeze fracture and scanning electron microscopy.

Each chapter begins with a useful outline of its contents. The reader is introduced to the techniques covered in the particular chapter, and in some cases a brief historical review of the subject is undertaken. This is followed by detailed descriptions of the various technical steps along with informative discussions of alternate methods in most cases. Each chapter is thoroughly illustrated and has a good, current bibliography. The overall quality of printing is high and the micrographs are well reproduced. The chapters on thin sections, cytochemistry and lectins each end with appendices of methods and recipes which will doubtless prove extremely popular with most researchers.

Of course, given the space restraints of the volume, it is not possible for each chapter to be absolutely complete. Different workers will undoubtedly notice different omissions. In the chapter on thin sectioning I was pleased by the detailed treatment of staining (although I believe that Reynolds should have been credited with the lead citrate procedure that was presented), but I felt that insufficient discussion was given to the value of low viscosity resins for embedding plant materials. In the same way, though I felt that the historical treatment of freeze fracture and the descriptions of standard freeze-fracture methods were masterful, I think that insufficient coverage was given to low-temperature, rapid freezing (Heuser-liquid helium methods) and the revolution that rapid freezing is currently causing in freeze fracture. Nevertheless, this is an excellent volume and should be available in all biological research libraries. Although the material is aimed towards plant biologists, it is of use and value to all biological electron microscopists. In spite of the volume's rather high price, I predict that many researchers will purchase it as a personal reference book. □

S.J. Kirchanski is a Postdoctoral Research Fellow and Lecturer at Harvard University Biological Laboratories, Cambridge, Massachusetts.

OBITUARY

L. P. Garrod

LAWRENCE PAUL GARROD, emeritus professor of bacteriology in the University of London died on 11 September 1979, aged 84. He was born on 7 September 1895. After school at Sidcot, Garrod's medical training at King's College, Cambridge and St. Bartholomew's Hospital was interrupted by service in 1917 and 1918 as a Surgeon Sub-Lieutenant, RNVR. At Bart's, his career as a student was outstanding and he won the Senior Scholarship in Medicine. In due course, after qualification, he became Chief Assistant to Dr Morley Fletcher and at that stage he seemed bound for the staff of the hospital as a consultant physician. But in 1925 he turned to pathology and then bacteriology, being appointed Professor of Bacteriology in the University of London in 1936, a post which he held with that of Bacteriologist to the hospital, until his retirement in 1961.

At Bart's he was a familiar and much respected figure. To the thousands of Bart's doctors who were taught by him during his time, he will probably best be remembered for his lectures and the associated practical classes at which he was a meticulous attender. A very clear logical speaker, his lectures were illustrated by a series of anecdotes and comments that held the attention of his students. To the hospital staff, his wide clinical and laboratory experience was always available. His opinion was always worth having and was much sought.

To a much larger audience, Garrod was known for his research and for his writing. Before the last war he became interested in disinfection and sterilisation, a subject that attracts the interest of few doctors, and he rapidly became one of the leading authorities in the country, with his name attached to one of the standard tests for disinfectants. This early experience stood him in good stead with the discovery of sulphonamides and then antibiotics, and a flow of papers began from his laboratory, many with his assistant, Miss Pamela Waterworth, dealing with chemotherapy in general and the properties and uses of each new antibiotic as it emerged. He played an important part in the Medical Research Council's trial of the penicillin treatment of subacute bacterial endocarditis, that was to set the pattern for many subsequent clinical trials.

As a writer, Garrod's contributions, both recognized and anonymous, were immense. He edited the *British Journal of Experimental Pathology* for many years and his close association with the *British Medical Journal* ran from 1931 until he died; he served on various committees and was at one time Chairman of the BMJ Journal Committee. When he retired from Bart's, the then Editor of the British Medical Journal, writing in the Hospital Journal, said that "it is difficult for me to say just how much we on the staff of the BMJ — and its readers — owe to Garrod. He has written book reviews, leading articles, annotations, answers to questions, all with effortless ease, precision of style, accuracy of fact, and a certain marked honesty that has been vastly refreshing."

Apart from his other writing, he was co-author of three books. First, before the war, *Recent Advances in Pathology* with Geoffrey Hadfield and then after the war, *Antibiotic and Chemotherapy,* first with Mary Barber and later with Francis O'Grady, and *Hospital Infection* with R.E.O. Williams and others. Retirement did not slow his pen, and he continued his work for the BMJ as well as answering the many invitations to speak that he received from this country, Europe and the United States.

Professional recognition came his way by honours and appointments. His views were sought as a committee member by the Department of Health, the Medical Research Council and World Health Organization. He examined for the Universities of London, Oxford and Cambridge. He was president of his section of the Royal Society of Medicine, Vice-President of the British Medical Association, President of the Institute of Medical Laboratory Technology, Consultant in Antibiotics to the Army, and on retirement, Honorary Consultant in Chemotherapy to the Royal Postgraduate Medical School. The University of Louvain conferred the title of Honorary Alumnus on him, Glasgow awarded him an Honorary Doctorate of Law and the Royal College of Pathologists elected him an Honorary Fellow.

R.A. Shooter

John Raymont

THE sudden death of Professor J.E.G. Raymont on 30 August 1979, at the age of 64, has deprived the world's community of marine scientists of a respected member and the field of zooplankton of one of its most distinguished students.

His interest in zooplankton began at University College, Exeter where he took first class honours in zoology, and was soon reinforced during 1937-38 when he held a Henry Fellowship at Harvard and spent the summer of 1937 at Woods Hole. After a brief return to Exeter as assistant lecturer he was appointed Lecturer in Zoology in Edinburgh in 1939. There, in conjunction with Sheina Marshall, F. Gross and A.P. Orr, a study was commenced on the effects on plankton and fish productivity of fertilising sea lochs, which was a precursor to marine fish farming in enclosed waters. During this period he met and married Brigit Sloan, who remained his constant companion at home and colleague at work in an enviably happy marriage.

In 1946 John Raymont moved back to the south to take up the Chair of Zoology at Southampton at the early age of 31. A generous grant from the Goldsmiths' Company enabled the purchase of the department's first boat, *Aurelia,* and his attention, after a brief period of working with other organisms, turned back to plankton. This was an era of public and scientific interest in the possibility of utilising plankton directly or indirectly as food organisms and he initiated long-term studies on the composition and physiology of zooplankton species. One of the researchers attracted by his studies in this field was a young Indian biologist, S. Krishnaswamy, who subsequently collaborated with John Raymont on several occasions. Partly through this connection and partly from international prestige gained as a result of his definitive work *Plankton and Productivity in the Oceans* (1963), John Raymont was invited under the auspices of the Indian U.G.C. and the British Council to advise on the establishment of the Centre of Advanced Marine Studies at Porto Novo. Thus began a long association with the training of oceanographers in many lands which culminated in membership of such bodies as the I.O.C. (Training, Education and Mutual Assistance), S.C.O.R., Nuffield Tropical Marine Biology Fellowship Panel and Chairmanship of the UNESCO Advisory Panel on International Marine Biology Centres.

At Southampton his academic career was crowned by appointments as Dean of the Faculty of Science 1961-1963 and Deputy Vice Chancellor 1966-68. His real ambitions lay, however, in a somewhat different direction and these were fulfilled in 1964 with the foundation of a department devoted to the study of marine science in all its aspects. Under his skilful guidance the staff of this multidisciplinary department were welded into a coherent and integrated unit in spite of their disparate research interests. The department quickly gained the international reputation which will live on as a monument to John Raymont's skills as a quiet unifier.

His international commitments, research culminating in some 60 papers, and administration came nowhere near exhausting his energies and over the years he was *inter alia* an adviser to the C.E.G.B. on matters relating to warmed water release, a member of the British National Committee for Oceanic Research and the Royal Society Pollution Study Group, member of the Councils of the Fresh Water Biological Association, Marine Biological Association and National Institute of Oceanography. As Chairman of the

Planning and Development Committee of the Wessex Regional Hospital Board and later as Vice Chairman of the Hampshire Area Health Authority, he was deeply involved in the expansion of hospital services in the area.

He was honoured by the award of the OBE in 1978 and earlier by election to a Fellowship of the Indian Academy of Sciences.

Marine scientists the world over will grieve the loss of a man who by quiet example was able to foster participation between research workers at every level, of different interests and from many nations.

A.P.M. Lockwood

Jack Tizard

PROFESSOR JACK TIZARD died in London aged sixty, on 2 August 1979, as a result of cancer found to be inoperable eight months earlier. So passed one of the foremost post-war research workers in mental retardation and child development.

Born a New Zealander, Jack graduated there and after overseas Army services undertook a postgraduate degree at Oxford where he met his wife. In 1948 he joined the Medical Research Council's Social Psychiatry Research Unit at the Institute of Psychiatry in London, and with his colleague Neil O'Connor was directed by Professor Aubrey Lewis into the neglected field of mental deficiency. He was appalled by the situation he found in hospitals for the mentally defective which in those days were used as places where socially disadvantaged young people could be put away. As a fearless man he said so, among other things challenging the medical model which was enshrined in the 1913 Mental Deficiency Act. Tizard and O'Connor set about showing that industrial habilitation leading to fairly rapid discharge from care was a viable possibility. Their pioneering studies on the mildly retarded were brought together in 1956 in an important book *The Social Problem of Mental Deficiency*.

However, there was more to do. There remained the moderately and severely retarded who were on the whole considered unemployable in even the simplest capacity. In a series of innovatory experiments it was shown that young adults with intelligence quotients in the thirties and forties could in certain circumstances learn, respond to incentives, retain their learning and transfer it to rather different situations. Others built upon and extended this work which cast new light on our potential for helping the more severely disabled, and their own potential for change.

Jack himself was ready to move on, and with the help of Dr Leslie Hilliard transferred a number of mentally handicapped children out of the Fountain Hospital and into a country house which resembled a boarding school. In 1961 he published *The Mentally Handicapped and Their Families* (with J.C. Grad) and in 1964 *Community Services for the Mentally Handicapped*. His belief that the quality of the social structure and organization of any community is a major determinant of development and behaviour of its members led him into the field of social policy.

In 1964 Jack was appointed to the first Chair in Child Development at the University of London; his outstanding contribution to the study of mental subnormality was acknowledged in 1968 when, jointly with Neil O'Connor, he received the Kennedy International Scientific Award, to be followed in 1973 by the annual research award of the American Association on Mental Deficiency. In the early nineteen-seventies, as Research Professor, he founded The Thomas Coram Research Unit, a unique multi-disciplinary establishment in which medical and a variety of social scientists collaborated in policy-oriented research projects. His erudition, high intelligence and unusually attractive personality ensured that he became much in demand for committee work. An excellent and incisive chairman, he was invited to act in several different capacities for a large number of organizations including the Social Science Research Council, the Department of Health and Social Security, the Association for Child Psychology and Psychiatry, the WHO Expert Committee on Mental Health, and for the British Psychological Society as president. He was appointed CBE in 1973.

Despite his generous contribution to committees, his research continued to flourish, and a steady flow of books and articles appeared over the years on a wide variety of topics relating to children. Jack Tizard was an example of that unusually effective individual, a radical thinker accepted by the Establishment.

In the final eight months, much of it in pain, Jack's bearing befitted his life. Fortitude, equanimity and hard work were his daily purposes; his courage was well matched by that of Barbara, his wife. Their long, happy marriage must have given both a background of security, and contributed in no small measure to the success each enjoyed.

To say of a man that "we shall not see his like again" is often a sentimental exaggeration; in the case of Jack Tizard, this is not the case. Those hundreds of us who knew him personally must feel a deep sense of loss at his passing.

Ann Clarke and Alan Clarke

Marcel Florkin

BORN in Liège, Belgium in the first year of the new century (15 August 1900), Marcel Florkin died in Liège on 3 May 1979. His early education and his medical degree were also at Liège, but thereafter he gained international recognition through his contributions in the areas of invertebrate physiology, comparative biochemistry and physiology, biochemical evolution, and origin of life, as well as through numerous roles in the development of international aspects of biochemistry.

Florkin's interest in physiology and biochemistry were increased by study in the laboratories of such eminent scientists as Joseph Barcroft, Richard Kuhn, Heinrich Wieland, and Edwin Cohn, following which he spent 36 years in teaching and research at the University of Liège, interrupted by visiting professorships at Duke University and the Universities of Washington and Oregon.

Perhaps the most compelling urge of his life in science was to extend his interests and contributions beyond ordinary limits, into broadly related areas of history, comparative aspects of physiology and biochemistry, evolution, and the nature of men who have contributed uniquely to the field of biological science. In addition to his numerous individual contributions, he undertook prodigious tasks of assembling as senior editor the world's contributions in multi-volume collections in various fields. Witness the ten volumes of *Chemical Zoology*, the seven volumes of *Comparative Biochemistry* (Florkin and Mason), and the 34 volumes of *Comprehensive Biochemistry* (Florkin and Stotz). The last five volumes of the latter series were devoted to the *History of Biochemistry*, his own contribution, and constituting written testimony to his critical and intimate knowledge of the great events in biochemistry. Included in his *History* is a remarkable collection of photographs of pioneers in biochemistry, itself a notable tribute to his perseverance and human touch.

Florkin contributed greatly to the role of biochemistry at the international level. In 1952 he became chairman of the International Committee of Biochemistry, and in 1955 was named the first President of the newly formed International Union of Biochemistry, following which he was instrumental in gaining admission of the newly formed Union into the International Council of Scientific Unions. As first Treasurer of the Union, the writer can attest to the diplomatic skills and warm spirit of negotiation displayed by Professor Florkin.

Marcel Florkin gained many honors and awards in his active life, including honorary membership in the Royal Institution of Great Britain and honorary fellowship in the Royal Society of Edinburgh. Yet in his busy life he maintained an active interest in the arts, and seldom missed an opportunity in his travels to find a moment for a visit to the local art museums to view their recent acquisitions. It is not surprising that he was devoted to family and close friends, who, along with many scientific colleagues, will miss his great warmth and human spirit.

Elmer H. Stotz

nature

21 February 1980

The Brandt report: better luck this time?

BLUEPRINTS for global survival have become an increasingly common fixture in popular debate over the past few years. Frequently presented with almost millenarian zeal, their appeal reflects an awareness that the growing interdependence of national economies has also nurtured potentially self-destructive tendencies of global proportions. The report* of the commission,headed by ex-West German prime minister Willy Brandt, suffers, like many of its predecessors, from a heavy overdose of idealism. But it deserves to be taken seriously, because it appears at a time when the stability of East-West and North-South relations is more fragile than for the past twenty years.

In one sense the Brandt Commission — officially known as the Commission in International Development Issues — picks up where the Pearson Commission left off ten years ago. At that time, the main question was the appropriate level of development "aid" which the rich countries should provide to the poor; Pearson's answer was to set a target (which few countries have lived up to) of 0.7% of gross national product. Since then attention has shifted from the level of aid contributions to the conditions under which development can take place. And one way to read the Brandt Commission's report is as a reply to recent vocal demands for a "new international economic order".

The commission's message is that the development of a strong global economy will be of mutual benefit to rich and poor countries alike; but that this will not be achieved merely through the unfettered workings of the global market-place. What is recommended is almost a formula for an international welfare state. Political institutions, the commission argues, need to be developed which will protect the weakest actors on the international scene; bring order to the financial chaos which would result if market-forces were left unchecked; and seek redistribution of global wealth through international taxation.

As a prelude to this strategy, the report repeats a by-now familiar litany of the sad state of the world we live in. Furthermore, many of the specific proposals also carry a familiar ring. Some have already been heard from numerous multilateral aid bodies. Others are more often proposed by the developing countries themselves, and their espousal by a commission calls both for a massive transfer of resources from the developed to the developing nations, and for a strict code of conduct to regulate the activities of multinational corporations. At present both are being resisted, the former most recently at the Delhi meeting of the United Nations Industrial Development Organisation (UNIDO), which ended in stalemate earlier this month. There are other, less familiar, suggestions. For example, the commission devotes a chapter of its report on energy. It recommends that there should be a global energy research centre, established under UN auspices.

But it is in the field of economic reform that the commission makes some of its most far-reaching, controversial, proposals. Here the commission is calling for no less than the restructuring of international financing mechanisms, particularly to cope with the cash surpluses arising from the oil revenues of the OPEC countries. One of the most significant aspects of the redevelopment scene over the past decade, it points out, has been a shift from government-financed aid to loans from private banking and commercial institutions, many of which have used such loans as a way of recycling OPEC deposits.

The commission argues that this massive recycling has created instabilities in the system which the private sector may prove inadequate to control; and that the massive debt burden already carried by many Third World countries will inevitably dominate development efforts for many years to come. Furthermore aid offered on commercial loan terms will tend to encourage relatively short-term development needs. But the commission is also critical of the stringent conditions laid down by the International Monetary Fund for loan assistance, arguing that these have often "tended to impose unnecessary and unacceptable political burdens on the poorest", and have led countries to prefer the private international banks.

The solution suggested by the Brandt Commission is to institutionalise ways of liberalising the international financing system. With respect to the IMF, for example, it suggests that the fund should "avoid inappropriate or excessive regulation" of the economies of the developing countries, and not impose "highly deflationary measures as standard adjustment policies". As far as the World Bank is concerned, the commission suggests, among other things, a shift to greater emphasis on programme loans, which would give a country flexibility in the methods it adopts to achieve a certain broad objective; it also suggests decentralising the bank's activities from Washington and giving a greater role in decision-making to representatives of the Third World.

Talking in realistic terms, any moves which would circumvent the role of existing financial institutions are certain to meet considerable opposition. Equally controversial is the suggestion for an international taxation system, all countries being assessed for their ability to pay into a general fund which would be made available for development purposes. The political appeal of such a system, possibly taking the form of a tax on international trade, is obviously limited; yet in his introduction to the report, Willy Brandt states that "by the end of the century the world will probably not be able to function without some practicable form of international taxation."

Those who followed last year's UNCSTD debate closely will not have missed the irony. For one of the major stumbling blocks in Vienna was the refusal of the developed countries to agree to the creation of a new fund, proposed by the developing countries to be based on contributions automatically assessed as a percentage of the trade in manufactured goods between rich and poor countries. It was merely agreed to establish an interim fund, based on voluntary contributions.

Indeed the commission's sketchy reference to the UNCSTD conference, grasping the inadequacy of its conclusions but providing little insight into why no more was achieved, merely illustrates the report's major weakness. For it is written more as a political manifesto than as a realistic framework for planning; but one that, given the growing attractions of protectionism and monetarist policies, lacks wide appeal at present. Furthermore the message that it is in the self-interest of the rich to promote the development of the poor inevitably invites the scepticism of those who would claim it as an expression of neo-imperialism.

But in one instance at least, the commission is correct. Suggesting that the industrialised countries restimulate their efforts to meet the aid targets to which many are already committed — and indeed to aim for 1% of GNP by the year 2000 — it adds that "what the neglect of foreign aid expresses is ultimately the lack of political priority attached to it." Next month in New York will see the pledging conference for the interim fund recommended by UNCSTD to finance new scientific and technological endeavours in developing countries, administered by the United Nations Development Programme. The event will indicate how much the main recommendations of the Brandt Commission have been taken to heart. Current indications are that total contributions will be less than initially hoped for although some countries — Britain excluded — will feel it appropriate to make reality of the Brandt Commission's analysis; and the acceptability of its message. □

**"North-South: A Programme for Survival" the Report of the Independent Commission on International Development Issues. Published by Pan Books, London, £1.95.*

High energy physics

Money may determine West Germany's view

IN the battle — imagined or otherwise — between the Hamburg laboratory DESY and the European subnuclear physics centre CERN near Geneva, Germany will give CERN priority, unless outside factors intervene. So Dr Günther Lehr, director for international cooperation of the German federal minsitry for research and technology, told *Nature* last week.

The pace of diplomacy over the issue between Italy and Germany is increasing. Italy was the first to raise doubts about German intentions. In the past few days the Italian minister of science, Vito Scalia, met Lehr with the chairman of the European Committee for Future Accelerators, Professor Marcel Vivargent, in Zurich — construed as neutral territory. And Professor Herwig Schopper, director of DESY and holder of all but Italy's vote to be CERN's next director-general, visited Scalia in Rome to clarify his position.

However, according to a Rome diplomat "it is still too early to say" if all the problems are solved. "Time has been useful" he said. "It has removed the cloud to a certain extent, there has been a clarification of problems."

Italy, which since the closure for high energy physics of its ADONE electron ring at Frascati near Rome has relied heavily on the CERN facilities at Geneva, fears competition between DESY's plans for an electron-proton ring, HERA, and CERN's plans for a large electron-positron ring LEP. The first stage of HERA, which would probably operate some years before LEP, would also be an electron-positron ring though at a nominal energy of 35 GeV on 35 GeV (see *Nature* 31 January page 420), below reach of the intermediate vector boson. Italy has perceived Schopper's potential appointment to the CERN director-generalship as a "Trojan horse" which would lead to the running down of CERN and the aggrandisement of the DESY laboratory in Hamburg.

But Lehr dismisses these fears. "DESY has risen in the last few years from a purely national laboratory to one of international importance" he said last week. "At first this caused surprise, and now there is overreaction."

Lehr is now convening a small group of scientific advisors to make recommendations, sometime this year, on spending in Germany on ten 'big projects', including LEP and HERA. Others include a new neturon source, equivalent to the UK Rutherford Laboratory's spallation neutron source now under construction, a European synchrotron radiation source as proposed by the European Science Foundation, improvement of the heavy ion accelerator at Darmstadt, and a deep drilling project to reach the Mohorovicic discontinuity, the 'Mohole' project dropped by the US some years ago.

Since Germany contributes 25% of CERN costs (the maximum allowed), the costs to it of LEP and HERA are roughly equal. But, said Lehr, "I would assure Scalia in Zurich that the LEP and HERA decisions would be independent. I cannot see us doing HERA instead of LEP. I rule out doing neither, but I see no basis for choosing HERA in favour of LEP, unless it is for reasons outside our control."

Such reasons might be objections to LEP from other member states of CERN. The Scandinavians particularly are thought to be cool to LEP (after all Hamburg is closer than Geneva) and the UK is likely to be in favour only if LEP is built slowly, to reduce annual costs.

However, cost is also a prime consideration in Germany, and Lehr does not know how much money will be available to him. Discussions are presently taking place on the science ministry budget for 1981, and it appears that there will be only "a relatively small increase" constrained by the international economic and political situation. There will not be enough money for all the big projects, but may be enough for more than one — perhaps 50 million DM per year.

"In these circumstances", says Lehr, "I cannot forecast my minister's reaction" to a proposal to go ahead with LEP and HERA together. This uncertainty leads directly to Italy's doubts. If Germany decided to go ahead with HERA before there is a decision on LEP (which will not come before the CERN council meeting of June 1981 at the earliest) would Germany be inclined to temper its enthusiasm for LEP?

Lehr regretted the Italian position on Germany and LEP "but in some respects I understand them, because DESY has been successful. CERN — I choose my words carefully — has been better at building superb accelerators than at discovering spectacular physics."

Schopper: CERN's next director-general?

Why should this be so? Because, suggested Lehr, the director general of such international bodies tries always to be on the safe side, to build an accelerator which runs on the touch of a button. "We should consider this matter" said Lehr, who is also vice-president of CERN Council, "and perhaps change this policy. We could build this smaller, cheaper, and take risks . . . . It will not be possible to build larger and larger particle accelerators: governments and other scientists won't accept it." Schopper, Lehr believes, would be a director general who would take imaginative risk, as he has done with DESY.

The Italian science ministry, however, told *Nature* last week that there could be no decision on the director generalship, Rome's main political weapon, before the June meeting of CERN Council, and that the time before then should be used to clarify the issues further. Lehr, on the other hand, insists that Schopper's early appointment as director-designate — the post is effective from January 1981 — would help to clear the ground for LEP. Strictly, a two-thirds majority is all that is required for a decision, but it is felt that unanimity is necessary on this appointment.

Scalia and Lehr appear to agree on one thing: the need for speed with LEP, if it is built. Lehr would like it built in five years rather than the seven which is current CERN thinking. This can be done within a 'constant' CERN budget of 620 million SwFr a year, says Lehr, if a minimum one-sixth version of LEP (with a maximum luminosity at 49 GeV) is adopted. Italy is in agreement with this. It would entail the same tunnel, but having fewer accelerating cavities so that the machine just reaches the mass of the intermediate vector boson, the Z_0.

Lehr sees US competition for the Z_0 as less of a threat than some: "John Deutsch has told me that his department of energy will spend some $300 million on Brookhaven National Laboratory, Fermilab, and the energy doubler, and Richter's Z_0 factory, so you can expect delays for lack of funds."

Rome does not regret the stand it has taken. A diplomat in the science ministry told *Nature* last week "our pressure has produced an increased awareness of the need for speed. The matter has been brought into the limelight." The diplomat "found room for agreement" in the language now used by the German delegates to the CERN Council. "We believe Germany is a very strong member of CERN, and wish it to ramain so."

Robert Walgate

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United States

USDA to study potential anti-social effects of sponsored research

FOLLOWING the US Department of Agriculture's decision to stop funding research that would throw farm workers out of their jobs (*Nature* 3 January 1980 page 5), Agriculture Secretary, Mr Bob Bergland, has now announced a study into the possible anti-social effects of all federally-funded research.

The task force that will undertake the study will concentrate on the $1 million which the Department spends annually on research at universities and land grant colleges into the mechnisation of agricultural production. This has been subject to criticism that led to the decision to halt new funds for such research.

In a speech to the staff at the Department's Science and Education Administration at Reston, Virginia, Mr Bergland repeated that he would no longer support research whose benefits would be restricted to a particular locality or social group, or which posed a threat to the natural environment or social community.

"In the specific case of mechanisation research, this means that we will not put federal money into research where — all other factors being equal or neutral — the major effect of that research will be replacing an adequate and willing workforce with machines", Mr Bergland said.

However he added that the economic incentives of the market-place should be powerful enough to enable this type of research to be funded by private industry, either by itself or with the help of state funds.

Mr Bergland said that this policy was consistent with the department's responsibilities to meet national needs, rather than those of a particular group. The government should not finance research whose beneficiaries could, in an extreme case, use it "to gain control of the farm-to-market structure, monopolise the sources of finance at every step, and increase their profits by selling what may well be an inferior product at a price that is insulated from competition".

He pointed out that until recently, agricultural research had been evaluated almost exclusively in terms of what the research promised in the way of volume productivity gains; too often potentially significant negative economic and social effects were not factored into the evaluation.

"The result was a skewed cost-efficiency statistic and distorted perspective. If this is not remedied in the research evaluation process, it will surely handicap our ability to meet changing national priorities", Mr Berland said. This was the reason for establishing a task force to look for research projects involving "the dubious use of federal money." □

New group to study cryptography policy

WAYS of resolving conflicts between the academic freedom of researchers working on computer codes and the interests of national security are to be investigated by a group that has been established by the American Council of Education, under a grant of $32,000 from the National Science Foundation.

The chairman of the study group will be Dr Werner Baum, dean of the college of arts and sciences at Florida State University. Dr Baum was previously chancellor of the University of Wisconsin at Milwaukee, where in 1978 a computer scientist, Dr George Davida, had a patent application for a new computer code blocked by the Department of Commerce following objections from the National Security Agency.

Dr Baum told *Nature* recently that he hoped the study group would be able to recommend policies and procedures which would both protect the legitimate academic and property concerns of universities and research institutions on the one hand, and the concerns of the US government about security on the other.

He added that this might involve changes in the law. In the Davida case, for example, which was successfully challenged by the university, Dr Baum pointed out that the NSA had no due process requirement covering demands that an individual should not publish the results of his research for national security reasons. Current law, he said, which goes back to the post-war McCarthy period, may turn out to be non-constitutional.

The ACE working group was established after NSA director Admiral Bobby Inman last year suggested a dialogue with the scientific community on conflicts between academic freedom and security issues. The group will include representatives of professional societies such as the American Mathematical Society and the Computer Society of the Institute of Electrical and Electronic Engineers, as well as legal advisers. It is expected to produce a report within a year.

Last year Admiral Inman was quoted as supporting legislation which would control research and the subsequent publication of results of "a central core of critical cryptologic information . . . that is likely to have a discernable adverse impact on national security." □

Battelle paints healthy picture of US R&D

IN contrast to the frequent complaints still heard about the health of US research and development, a surprisingly lively picture of the current situation is presented in the latest annual analysis by the Columbus Laboratories of the Battelle Memorial Institute. Taking R&D expenditures across the board, Battelle estimates that these will grow by 19.7% in 1980, to a total of $61.8 billion. Within this total, industrial funding is expected to grow faster (20.9% increase) than federal funding (19% increase); but even given a predicted cost increase of 12%, this still implies a 7% real growth in expenditure, reaching a new peak in total real funding of R&D, the report says.

Despite the unfavourable economic climate, the Battelle report says that there are many intangibles, "particularly the reawakened feeling on the parts of many decision makers that R&D is essential to our long-term economic health — that favour greater-than-usual increase in support for research. The report says that its analysis indicates that the upward movement established over much of the past decade in R&D expenditures is likely to continue, with the 1980 figures leading to an average increase over the past seven years of 3.3% in real terms. It estimates that during the decade of the 1980s real R&D activity will increase at an average rate of approximately 3%.

The major sector to indicate increases in both funding and performance of R&D in 1980 is expected to be industry. In contrast, academic and other non-profit institutions are expected to register small decreases in the percentage of support and performance (3.5% and 15.1% respectively); but there will still be significant increases in the total activity of each (1.3% and 17.6% over the estimated 1979 figures).

The report says that therefore in spite of inflation and the desire of the Carter administration to balance the budget, there are signs of greater support for research. It points out in particular that industrial support is growing in fields affected by regulations and in those fields most directly influenced by the need for more energy-efficient products and processes. □

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Finland

Public confidence in science is reviving

LAST week, Finland's Swedish-language technological society, the Tekniska Foerenigen I Finland (TFIF) held a panel discussion on the country's energy policy as part of the centenary celebrations of the society's foundation. According to Erik Moring, the president, of TFIF, the holding of this discussion, between five of the country's leading experts from both the government and private sectors, reflect the general theme of the year's celebrations — to show the country that scientists and technologists are not "some sort of monster."

The TFIF celebrations, with their strong emphasis on the best use of Finland's natural resources for the good of the country as a whole, coincide with Finland's current "environmental year", and a general swing of public opinion away from the strongly anti-science bias of the mid and late 1970s. On 8 February, the Social Democrats (the largest single party in the Finnish parliament) called for a major investment in science and the creation of 300 new research posts. A few weeks previously, Finland's highly charismatic president, Urho Kekkonen, called for a reassessment of R&D investment and the expansion of research facilities — the first time that the president called so directly for a boosting of science since 1964.

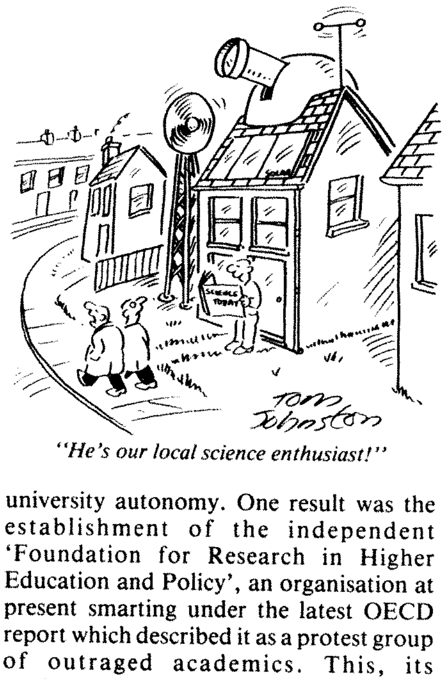
The result that previous appeal was a major reorganisation of research planning, leading in 1969, to the foundation of the Academy of Finland, which, in spite of its name, is essentially a system of research councils and of government science funding. When the Academy put forward its five proposals for increased government science funding as "areas of emphasis", these had a strong bias towards the social sciences: environment, work and working conditions, public health, basic necessities of the population and living conditions, and the implementation of democracy and equality. It was this last project that, in the opinion of many Finnish scholars, led to the breakdown of public confidence in science planning.

Undoubtedly the project did provide a vocal focus for grievances. According to its opponents, the results of the research were widely politicised by the left. A nationwide debate sprang up in the press causing considerable disillusion among the public. The academic professions, which increasingly tended to range themselves against the Academy, had, however, other complaints.

During the past 25 years, Finland's general policy of decentralisation has increased the number of the country's universities from two (Helsinki and Turku) to 18 (the last of which, the university of Lapland, was founded in 1979). Some of these are genuine new foundations, others are upgraded/former specialist or teacher-training colleges (e.g. the University of Jyvaeskylae). This proliferation was, to a large extent, necessary to prevent a permanent brain-drain to the south-west of the country.

However the expansion has left Finland, like many industrialised countries, with the problem of a very slow turnover of research staff in universities and few opportunities for new graduates who wish to stay within the Finnish academic structure.

An even more bitter cause for resentment, however, was the government regulation of the 1960s codifying and standardising the granting of academic degrees. This was, not surprisingly, seen as an encroachment on academic liberty, particularly since Finnish tradition followed the 19th century German ideal of university autonomy. One result was the establishment of the independent 'Foundation for Research in Higher Education and Policy', an organisation at present smarting under the latest OECD report which described it as a protest group of outraged academics. This, its spokesman, Dr Juha Vuorinen told *Nature*, is simply no longer true. "We are now cooperating much more (with the government) and the political issues are not so hot. What we are trying to do now is to influence the government to give more money to research. Research must be led by experts, not bureaucrats".



"He's our local science enthusiast!"

Certainly there are fields in which Finland is capable of taking a lead position. One is nitrogen fixation, traditional since the time of Professor Virtanen, who received the Nobel Prize in 1945. Another is the ultra-cold laboratory of the Helsinki University of Technology at Otaniemi. This laboratory was established by Professor Olli Lounasmaa in 1975 on the principal that low temperature physics is one of the frontier areas still within range of the Finnish budget. At present the laboratory holds the record of 50 nano-Kelvins (using a copper nuclear spin system). One byproduct of this work is the development of SQUIDS (superconducting quantum interference devices) to produce magnetic shielding. A magnetically shielded room is now under construction and an international seminar is to be held in May for potential users of what Professor Lounasmaa describes as "this rather expensive facility which will incidentally, be paralleled only by one under construction in Berlin."

Professor Lounasmaa's expertise in planning the laboratory has led to his being appointed head of a new working group to advise the government on the funding of basic research. Finland, he told *Nature*, has a very small bureaucracy. A scientist can therefore hope to obtain funds without too much red tape. Also, although the country has no long tradition in science, the general public, he says, has on the whole a high esteem for science. The disillusion of the last few years is not, he feels, significant in the long run.

Certainly one can find numerous examples in Finland of public confidence in science. Last autumn, the leading Finnish daily, *Helsingin Sanomat* celebrated its ninetieth anniversary with a special series of articles on science — which, in the opinion of Dr Elizabeth Helander, research director of the Academy of Finland, is the principal cause of the recent public change of heart. Some such gestures have involved considerable sums of money. In 1967, to mark the golden jubilee of the country's independence, the Bank of Finland set aside securities worth 300 million FM ($45 million) whose interest would be used to fund loans for R&D expenditure in the private sector. Some considerable financial daring is also involved in two new publishing projects. One, proposed by the Academy of Finland, is the publication of a new popular science journal which is expected to become financially independent within three years. The other is the publication of a five volume full colour encyclopaedia of Finnish wild life (the first ever such work), which is expected to sell 10,000 at 1600 FM ($220) the set. In a country of 4.5 million inhabitants and with virtually zero prospects of foreign sales, these figures assume that one household in ten throughout the nation contains at least one science enthusiast. **Vera Rich**

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NEWS IN BRIEF

East-West "persecution" row at Hamburg Forum

USSR Academician Nickolai Blokhin has accused the US and UK of "trying to make a 180 degree turn" in discussing international scientific cooperation and "interfering in the internal affairs of the Soviet Union". Speaking at the Hamburg Scientific Forum this week, Blokhin, head of the Soviet delegation and president of the Academy of Medical Sciences of the USSR, accused them of "trying to be teachers of other countries when no-one asked them to". He was reacting to strong appeals from Lord Todd, head of the UK delegation and president of the Royal Society, and Dr Philip Handler, president of the National Academy of Sciences, on behalf of persecuted colleagues. Lord Todd cited the Soviet Union's behaviour in blocking invitations to scientists to go abroad, and the harsh treatment meted out to scientists "for comparatively minor disagreement with authority".

"Does the Soviet government yet realise", he said "the resentment this causes amongst young scientists? I call on our Soviet colleagues to impress on their government the urgent need for change".

Dr Handler stressed that many Soviet scientists were victimised owing to their wish to monitor the Helsinki Accords. There had been, he said "a spontaneous upswelling of reaction in the US." His delegation was present in Hamburg in spite of many high level scientists' appeal for a boycott.

Blokhin did not answer the charges specifically, but harangued the Forum with a long list of Soviet scientific achievements which he said the US and UK were trying to deny. It had been expected the Soviet delegation would be led not by him but Dzherman Gvishiani, deputy head of the State Committee for Science and Technology.

US probes illicit Soviet technology sales

The US Department of Commerce is currently investigating numerous cases of illicit sales of computer, laser and transistor technology to the Soviet Union, according to a document leaked to the *Los Angeles Times*. Although black market sales in high technology to the USSR and the socialist countries has existed for years, this is the first time the government has attempted to document its extent and significance. Citing such procedures as the creation of dummy firms, hand carried shipments without export licenses, and shipments made to illegal destinations, the document lists more than a dozen US companies who have engaged in this profitable but shady trade. Included are such small companies as I I Industries and Kasper Instruments in California's Silicon Valley, which pleaded guilty in 1975 to diverting $330,000 of semi-conductor equipment to the USSR by way of dummy firms in Canada, and Information Magnetics Corporation of Goleta, California, which shipped $880,000 worth of computer supplies to Bulgaria through a UK subsidiary. Larger firms named in the document include Texas Instruments of Stafford, Texas, Tektronix Incorporated of Beaverton, Oregon, and Hewlett Packard of Palo Alto, California.

California gives major boost to solar energy

A major boost to the US solar energy industry has been given by a ruling from California's Public Utilities Commission that the state's four largest publicly-owned utilities must finance the installation of solar water heaters in 175,000 homes over the next three years.

The utilities — Pacific Gas and Electric Co., Southern California Edison Co., Southern California Gas Co., and San Diego Gas and Electric Co. — have been told to produce plans within 60 days for various types of low interest loans to solar-home owners.

Industry officials estimate that the commission's ruling will increase by more than five times that number of homes in the US with solar-assisted water heating. The plans still require further approval by the commission, and will be the subject of public hearings, but the PUC has indicated that it intends to achieve a major break-through in the acceptance of solar power by consumers.

"Certainly there isn't any question the PUC order is the biggest thing to happen to solar power", said Mr Edward A Myers, vice-president of Southern California Edison. Another utility official claimed that, as far as solar energy was concerned in California, "whether it is economically competitive really doesn't matter any more".

Average cost of installing the solar heaters is expected to be about $2,500 for each house for equipment which would provide 50 to 70% of the energy needed for hot water.

First geothermal plant in Holland

The first geothermal project, part of a larger 32 million guilder energy research and development programme has been started in Holland. Fifty houses in Spijkenisee, a Rotterdam suburb, will be connected in 1982 to a heating system which will circulate water at 100°C from 3000 metres underground. A unique aspect of the system is an interim storage procedure that will hold the water at a depth of 600 metres during the summer months when demand is less. An estimated equivalent of 12-25% of Dutch natural gas reserves is available in geothermal energy. The most favorable source is in the south of the country where studies carried out since 1974 have shown gradients of 40-50° per 1000 metres of depth. **Caspar Schuuring**

US pays radiation exposed veterans

Nineteen war veterans who were deliberately exposed to radiation from bomb tests in Nevada in the 1950s as part of a programme to show that the US fully intended to use its nuclear capability are receiving disability payments for cancer. But the great majority of 493 veterans who have filed claims for cancer contracted as a result of the tests have been turned down. Ten cases were awarded benefits and an additional nine were won on appeal but the Army denied the remaining 386 applications. Other cases are still pending.

UK nuclear inspectorate understaffed

The UK's Nuclear Installations Inspectorate is facing increasing difficulty in meeting its obligations because of government spending cuts. Present staffing is 15% below full complement (17 vacancies out of 104 positions), and recruitment has proved difficult because industrial salaries are 30% higher and because of the Inspectorate's threatened move from London to Lancashire. In addition there are fears that the Health and Safety Executive may be forced to impose non-nuclear duties on its nuclear staff because of the impact of recent budget cuts. Nuclear inpectors are highly specialised and must be qualified engineers with 10 years' industrial experience. Mr Roger Gausden, Chief Inspector, has admitted in the NII's biennial report that "it has not proved possible to recruit the required additional staff to deal with all the tasks facing the Inspectorate".

University of Edinburgh rescinds "six year rule"

The University of Edinburgh has rescinded its rule preventing the rehiring of untenured members of research staff after six years. Citing opposition to the "rigidity" of the rule, a report by a university working party last week said that "research staff are in the best position to make the decisions individually". The report cautions, however, that heads of departments should not make any "unjustified commitments". The "six year rule" has been rescinded "despite the fact that there now appears to be a tendency to adoption of a similar rule by some research

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councils". The working party, chaired by Professor A G Mackie, surveyed the university non-tenured research staff and found that only half obtained permanent jobs on completion of their contracts, while 11% were unemployed and 32% had moved to other temporary jobs.

UNEP shames Mediterranean countries to pay bills

A MILITANT speech by United Nations Environmental Programme deputy director, Peter Thacher last week (*Nature*, 14 February page 613) has produced promises of payment by the delinquent participants in the Mediterranean clean-up programme. Italy has promised its $745,000 arrears by April, Spain its $412,000 payment by December and France its remaining $573,000 "sometime this year". In addition cheques which have not arrived from Turkey, Yugoslavia and Monaco will be traced. The pollution programme will be forced to close in seven weeks if part of the money is not made good. In another major result at the three day meeting in Barcelona, Algeria signed the 1976 Barcelona pollution accords leaving Albania and Turkey as the only non-signatories. Algeria's signing signals important Third World support for the clean-up programme, expected ultimately to cost $15 billion over 10-20 years.

France and India sign new technical agreement

FRANCE and India have signed seven new protocols for technical and economic cooperation which include research in renewable energies, oceanographic technology, and the development of semi-arid regions of the left bank of the Rajasthan Canal and the Bundelkhandmarea of Uttar Pradesh. Signed by French President Valery Giscard d'Estaing on his visit to India last month, the protocols will also attemt to solve the long standing problem of Franco-Indian cooperation — the inability of India to make use of French credits because of the high cost of French equipment and technical service. **B. Radhakrishna Rao**

Sri Lanka to set up basic research institute

SRI LANKAN President J R Jayawardene announced last week to establish an institute for basic research to be housed at a site near Colombo by November of this year. The culmination of nearly a year of discussions between the President, local scientists, and several expatriate Sri Lankan scientists, the institute will emphasise advanced study in all branches of fundamental science. Professor N C Wickramasinghe, head of the department of mathematics at University College, Cardiff, UK, has been mentioned as a candidate for director of the institute.

FEATURES

Papua New Guinea (PNG) is one of the world's least "developed" countries. A third of its three million people have only emerged from the neolithic age over the past 40 years. The people are divided by mountain ranges reaching 4,700m, torrential rivers, forests, ravines, seas, malarial swamps and language — more than 700 are spoken. But in the five years since independence from Australia, the government has launched an ambitious Improvement Plan, under which western science and technology are being introduced enthusiastically. All projects are funded by the National Public Expenditure Plan, which absorbs 21% of all spending. The main national aims are equal distribution of development among a population that is 85% rural and largely dependent on subsistence farming and a reduction in the number of western expatriates on whom development still largely depends. Both aims are meeting with mixed results, as **Tony Ades** reports

Appropriate technology:

PAPUA New Guinea's Eight Point Improvement Plan calls for self-reliance, less dependence for its needs on imported goods and services. Most conspicuous among imported services are those of the 4,200 expatriates employed by the government. While nearly all political posts are filled by nationals, expatriates have a dominant role in formulating policy and planning its execution.

Since self-government in 1973 the number of expatriates has fallen from 50,000 to 30,000, partly as a result of the localisation programme, the replacement of expatriates by nationals. In the public service, the proportion of expatriates has dropped by 10%, but there seems to be no hurry to enforce the planned 4% reduction per year because the number of qualified nationals is not increasing as fast as the nation's need for skilled administrators.

There are sound economic reasons for localisation. The average expatriate costs the government four or five times as much as the average national, and even at the same level the expatriate will earn between two and three times more. Also, their incomparably higher standard of living creates unfulfillable hopes in the local population. However, unlike most developing countries, PNG had no western-trained elite to take over at independence. Furthermore, the sheer inaccessibility of much of the country has brought education very unevenly. In the highlands, where many gratefully recall how the first Europeans ended interclan fighting after the Second War, people are inclined to believe that localisation has been going too fast, leaving them permanently disadvantaged to the coastal peoples whose longer contact with the West and higher levels of education give them disproportionate political power.

Of all government services it is in fact education where localisation has been most dramatic. By 1972 all primary school teachers were nationals. Since then the number of teachers has doubled to 10,000. And although 60% of all children now attend primary school, there has been a serious fall in standards that will take many years to work itself out of the system. In the meantime, localisation of secondary school teaching is going ahead.

The standards problem is most severe in science and mathematics. Complaints that young men and women training to be Health Extension Officers or agriculture technicians cannot calculate drug dosages or areas are commonly expressed, and at all higher education institutions students take remedial maths courses that may delay their regular curriculum by up to a year. Whatever the economic and political advantages of localisation, PNG's ambitious plans for development demand so much gathering of data on every aspect of the land and population, and so much administration, that many are asking whether localisation too early has actually increased dependence on expatriates.

Tony Ades has a Nature writing fellowship and is currently at the PNG Institute for Medical Research, on leave from Sussex University, UK.

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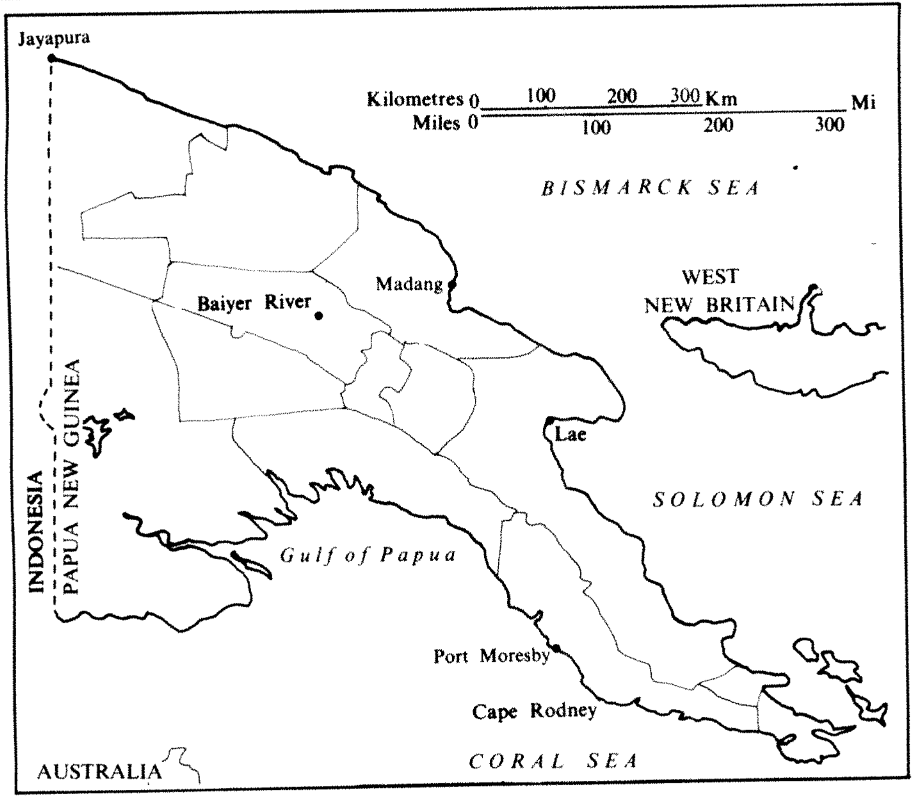

Papua New Guinea's view

An expansion of education and a consequent fall in average standards is not unusual in developing nations. In PNG it has lead to demands, particularly from expatriate education planners, that a more merit-oriented system be introduced. At present, selection from one level of school to the next is based on a quota system biased toward children from lower quality schools and poorer regions. This policy is typical of the government's determination to redress regional imbalances. The government is equally unlikely to yield to suggestions that it drop its plan for 100% primary school enrolment by 1990, on the grounds that vitally needed extension services in health and agriculture are seriously hampered by illiteracy.

The concern for equality, rather than higher levels of education for a few, was based on the idea that PNG would adopt small-scale, labour intensive modes of development. At the time of independence there was a corresponding enthusiasm for "appropriate technology". But the Village Task Force, set up in 1974 to carry this through to the rural people, seldom progressed beyond the theoretical stage. Six years later, the South Pacific Appropriate Technology Foundation (SPATF) acts as an information clearing house and sets up small projects like village water supplies, but is still very limited in its capacity to create rural employment. Instead, SPATF has been set to work on the urban unemployment problem, and devises pilot projects such as furniture making.

Its research arm, The Appropriate Technology Development Unit at the University of Technology, Lae, is also more oriented towards hardware. It has designed a hydraulic ram pump that can be built with a drill press rather than an entire machine shop and is currently testing mini-hydroelectric schemes. The newly appointed Vice-Chancellor, Professor Alan Mead, has promised to involve more of the somewhat reluctant faculty in this type of work, but there exists as yet no channel by which rural needs and technical possibilities can meet each other.

SPATF's explicit policy of "not telling people what they want" demands that people have some idea of what is available. It has always been hoped that the increasing number of school leavers, teachers, extension workers, and even some university graduates would return to their villages and catalyse development. But those few who try are often rejected by the village elders. Extension workers are now generally posted away from their own villages, losing the advantages of shared language and culture. On top of this, a western education, even if below western standards, does not prepare one for a return to a subsistence lifestyle. A student at the University of Technology told me that he might eventually go back to his village, but only when electricity had reached it and when television broadcasting had begun. Then he would help the village develop by selling and servicing television sets. Such views are not unusual.

The fact is that the "appropriate technology" emphasis on locally available materials and skills, and on the natural evolution of simple technologies at the hands of the craftsmen who use them, is scarcely relevant in a country with no traditions in metal, cloth or draught animals. For many the first wheel came on an aeroplane. The arrival of science and technology has been headlong: generally it is capital intensive and oriented to a high return. Papua New Guineans have welcomed sophisticated telecommunications, the F-28 jets flown by Air Niugini, and the highly sought after four-wheel drive land vehicles. And at the government level there is certainly room to doubt the commitment to appropriate technology. In spite of the recommendations in its own national paper to last years United Nations Conference on Science and Technology for Development, PNG operates no assessment or vetting of imported technical hardware.

On the other hand, in the field of energy, PNG enters the technological age with more foresight than any developed nation, and with a comprehensive policy aimed at self-sufficiency through renewable resources. The present expansion of the road system and the growing monetarisation is forcing up fuel imports at 8% a year. To counter this the initial step is to be a cassava-to-alcohol conversion plant in the Baiyer River area of Western Highlands Province. The 2 million litres produced each year will be mixed at 15-20% with motor spirit. The plant will be in a remote area not only to bring rural employment, but also to offset government subsidies for fuel transport costs, which run to K3.50 ($4.62) per gallon in the most inaccessible regions. (One PNG kina is worth $1.32.) If this conversion plant is a success, more will be built. It is hoped that the use of alcohol fuels will eventually reach a level that would justify the import of cars that run on 100% alcohol. At that point large scale wood-to-alcohol installations would be built, drawing on waste from forestry operations.

PNG has so far decided against rural electrification. Other aspects of the energy policy include pilot projects for charcoal production, wood pyrolysis, firewood cropping, together with research into solar air conditioning, sago to alcohol conversion, and biogas plants. Meanwhile, several small diesel generators are to be replaced with photovoltaics. The entire programme, the brainchild of Dr Kenneth Newcombe at the Department of Minerals and Energy combines all levels of technology with a sensitivity to rural skills and needs. It is also an outstanding example of how much scope expatriate scientists and economists have in moulding the course of PNG's development towards, paradoxically, self-reliance.

While many components of the energy policy accord with official approval of small-scale industry, and are therefore consistent with the educational system's stress on quantity rather than quality, the course of development appears to be moving in the other direction, calling for more skilled management than is available. A shift away from smallholder projects is becoming evident. For example, cattle smallholdings are to be replaced with ranches owned by entire clans, worked by hired labour and large enough to pay the services of an expatriate manager. A flagging smallholder rubber development at Cape Rodney, Central Province, is to be revitalised by the addition of a central estate. The World Bank's Southern Highlands Development scheme also opts for nuclear estates. The Baiyer River alcohol plant will be supplied with intensively farmed cassava. The rice and sugar projects now under consideration not only have no place for smallholders, but are on a very large scale indeed.

The causes of this trend go deeper than economies of scale and the unstable output of smallholders. Officials concede that there has been insufficient effort to make the smallholder self-reliant. Extension officers have arranged for bank loans to farmers and taken most of the decisions to ensure that the loans are repaid. Effectively the smallholder is turned into a labourer on a government-managed farm. On top of this is the difficulty of extension work among a largely illiterate population with a chronic shortage of staff. Yet the government is committing only K300,000 ($396,000) a year to adult literacy programmes. Moreover, Rural Development Assistants, who comprise the vast majority of agricultural extension staff, are to be phased out altogether. They will eventually be replaced with smaller numbers of better-trained staff, but an increasing proportion of students graduating from agricultural colleges go into private business for themselves.

Eventually, the educational system will probably iron out the currently erratic course of development at the rural level, if, that is, its western orientation does not promote dissatisfaction with rural life. However, it may be perpetuating the lack of local expertise which recently lead the Prime Minister Mr Michael Somare to try to drum up Australian investment. PNG is not at the mercy of foreign capital. Its laws clearly demarcate the areas in which investment is welcome, and it regulates the form that foreign business activities can take. But critics point out that this can only serve to amplify the gap between the still expanding subsistence population and the wage sector, and further disrupt the former.

In addition to this, the growing indigenous commercial interests, with their increasing political influence at provincial level, are also aiding the push towards a more capital intensive wage sector and away from subsistence and smallholder farmer. A related danger is that provincial governments are threatening to make their own arrangements with foreign companies, but have less ability to ensure favourable terms than the national government.

In accordance with its stated policies, Papua New Guinea is slowly achieving self-relieance as a nation, and redressing the inequalities between its regions. The question is: can it at the same time bring self-reliance and equality to its people? □

Depending on foreigners for self-reliance: expatriate manager (right) with cattle smallholders rigged out in clan gear

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Eric Ashby (right) looks at a recent report on public participation in technology decision-making in OECD countries and argues that better public information could reduce disenchantment with representative democracy

THE WINDSCALE enquiry in Britain and the Mackenzie Valley Pipeline enquiry in Canada were highly publicised experiments in participation. They were responses to a demand which has now become insistent, for "a greater degree of public accountability, freer public access to technical information, more timely consultation on policy options, and a more holistic approach to the assessments of impacts", to quote a report on participation from the Science Policy Division of the Organisation for Economic Co-operation and Development.

The report, *Technology on Trial*, by K. Guild Nichols, published in Paris last year, is a clear and useful account of the present state of the art of participation in OECD countries. The important point Nichols makes is that this demand is a symptom of a much deeper social disturbance — a disenchantment with the whole process of representative democracy.

The public elect people to represent their interests and straightway distrust them. Through the mass media self-appointed leaders who claim to represent the public interest can bypass the traditional hierarchy of procedure and appeal direct to the people over the heads of legislators. Nichols quotes Paul Valery: 'all politics is based on the indifference of most of those concerned, without which politics would be impossible'. If Valery was right, we are in for trouble; for concern, as measured by the number of people prepared to vote (for instance) in the referendum on atomic power held in Austria in November 1978, is spreading, and indifference to such great issues can no longer be assumed by those who make decisions.

Governments in pluralistic democracies are embarrassed by this surge of interest; as indeed they need to be, for this desire that the general public should participate in the decisions that affect their lives is undeniable in theory and confoundedly perplexing in practice. There is no such thing as monolithic public opinion. Even groups of people who agree about what is in 'the public interest' may do so for different reasons: one because he genuinely fears a nuclear economy; the other because he doesn't want a power station to spoil his view. The utilitarian calculus — that a social welfare function is the sum of a multitude of individual welfare functions — is a discarded and useless concept. The assumption, accepted for a long time by the man-in-the-street, that major

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Participating in planning: the public needs better information

technological problems can be left to experts to solve, is no longer valid. If we want to remain a pluralistic democracy, we have got to invent reliable machinery for public participation.

Nichols' essay describes how information on technological issues is conveyed to, and elicited from, the public; and he describes how policy makers are informed and advised. He draws examples from several countries: Britain, the US, Canada, Scandinavia, Germany, Austria, France. He picks out the difficulties and displays them clearly. How can an unofficial group of citizens get access to the necessary information? Where can they raise the money to dispute a governmental policy? How can state officials, promoting what they earnestly believe is the public interest, be protected from vexatious, and sometimes mischievous, obstruction from groups acting out of self-interest? What are the comparative merits of agencies, legislative hearings, commissions, and the courts, as ways to resolve conflicts of values?

Nichols gives an accurate and objective summary of recent attempts in OECD countries to reconcile the conflicts of fact and value which are inevitable when nations have to make decisions about exploiting the environment. In just over 100 pages he could not be expected to cover the whole range of social experimentation that has been tried. This range has been classified, somewhat tendentiously, by S.R. Arnstein (in *The Journal of the American Institute of Planners*, xxxv, 1969, p.216) in six categories, grouped in pairs: (i) manipulation and (ii) therapy (designed to 'cure' the participants of what the proponents of a scheme deem to be ignorant prejudice); (iii) providing information and (iv) consultation (deemed by some participants to be 'tokenism'); (v) delegated power to participants and (vi) citizen control (the two categories which Arnstein is prepared to regard as 'partnership'). Categories (i) and (ii) have been rejected by the lobbies which give themselves the duty of scrutinising the impact of technologies on the environment. As machines for public participation they are obselete. Category (vi) would be regarded, by all but the most fanatical opponent of representative government, as unrealistic. Categories (iii) and (iv) are acceptable to some lobbies, and they could be made more widely acceptable if they were taken more seriously by governments.

For models of intelligent consultation combined with masterly presentation of facts we have to turn to Canada. Two outstanding examples are the reports of Mr Justice Berger on the gas pipeline from Alaska (summarised by Nichols); and the outstanding report from the Ontario Royal Commission on Electric Power Planning (*A Race Against Time: interim report on nuclear power in Ontario*, Toronto, 1978). The explanation of the CANDU fuel cycle in this report is a brilliant example of interpretation to the layman of the issues he must understand before he can play a useful part in any process of participation.

The corporation that generates electricity — the Ontario Hydro — has no statutory requirement to involve the public in its planning processes. Nevertheless, since 1972, the corporation has involved about a hundred people at head office and in its regions, holding public hearings, setting up citizens' committees, and listening to anyone who cared to talk to them.

Whether this has educated the public to understand the issues better is something that cannot be proved; but there is no doubt that the public have educated Ontario Hydro, and the Commission.

This, however, falls short of partnership in decision-making, and there are very few examples of the delegation of some power to the participants, Arnstein's category (v). Only one successful example of that is on record. It is to be found in a report prepared for the Electric Power Research Institute in California (*Proceedings of a Workshop on the Measure of Intangible Environmental Impacts*, EPRI Special Report, Palo Alto, 1976). The San Diego Gas and Electric Company had to site a new power station in southern California. They invited the public to set up a committee of their own to examine alternative sites, provided them with financial help and expert advice, and left them alone to hold their own public hearings and to make a recommendation, which the Company accepted.

It is unlikely that participation in Britain will go beyond the levels of Arnstein's categories (iii) and (iv) in the foreseeable future; but if bodies like the Central Electricity Generating Board and the National Coal Board bring imagination and genuine enthusiasm to their schemes for providing information and for seeking consultation, the benefits even of limited participation could be considerable. First, it could restore some confidence in the judgement of those who inevitably have to make the final decisions (the process of decision-making is as important for society as is the wisdom of the decision itself). Second, it could encourage a sense of responsibility among the so-called public interest lobbies.

More participation will not necessarily clarify the issues themselves: indeed one of the surprising results of a massive experiment in public education about nuclear power in Sweden, financed from public funds and involving some 80,000 members of the public, was that at the end the number of people who felt unable to decide either for or against nuclear power *increased* from 63% to 73%. This is not — as some government officials asserted — a sign that the experiment failed; it is, rather, a sign that more people than before are aware of the immense complexity of the problems facing their representatives in government. This could increase public sympathy with decision-makers; and this in turn could help to turn the tide of disenchantment with representative democracy.

Our educational system must bear some of the blame for this disenchantment. In the last thirty years the impact of science on society has gone up by many orders of magnitude; but science at the popular level (for those who are not going to become professionals) is taught in very much the same old way.

We have not paid enough attention to telling people what they need to know about science if they are to perform their civic duties. (Often we tell people too much: Nichols quotes Thurber's little girl: "This book tells me more about elephants than I ever wanted to know".) The American science writer Philip Ritterbush put the challenge this way: '. . . scientific understanding of the public, rather than public understanding of science, might become the frame for discourse'. ☐

Lord Ashby is Chancellor of Queen's University, Belfast, Northern Ireland, and a fellow of Clare College, Cambridge, UK.

CORRESPONDENCE

Biotechnology and scientific collaboration

SIR, — In a recent issue of *Nature* (10 January, page 125) I was pleased to note in Robert Walgate's article the inclusion in the proposed EEC programme on biotechnology of "development of reactors for reactions at a hydro-organic interface", since our Biotechnology Unit has been responsible for several PhD theses and published papers related to this topic. The unit, set up several years ago, did effectively organise collaboration between biochemists, chemical engineers and microbiologists in this and other fields.

It was therefore surprising to read on the following page that "Lilly's group is unique in the UK in combining chemical engineers and biologists". While this group has indeed been pre-eminent in developing recognition of enzyme technology in this country, Dr Walgate could have ascertained that it was not unique, by reading the 1976 Report to the SRC and Institution of Chemical Engineers by A.N. Emery which cites at least four UK universities where such collaboration occurs.

A point of more concern is evidence of conceptual isolation of food science from biotechnology. Both involve application of the same basic disciplines of biochemistry, microbiology and chemical engineering and are similarly concerned with the provision, processing and utilisation of natural products. Both involve enzyme technology. This isolation is surely based more on territorial tribalism among applied scientists and their professional organisations than on a rational approach to the subject matter.

Now that the universities have entered lean years, where the relationships between the financial bones and the semantic skins are more carefully scrutinised, it is to be hoped that the various bodies concerned with biotechnological planning will not take too narrow a view of existing and potential assets.

Yours faithfully,

J.A. BLAIN

University of Strathclyde, Glasgow, UK

FAST's biotechnology programme

SIR, — Your issue of 10 January focusing on biotechnology was timely and relevant, in emphasising that "the middle-term problem rests with governments", and that "this is precisely the moment when patterns of research will be formed which will ultimately shape lives". The Commission's "FAST" programme (Forecasting and Assessment in the field of Science and Technology) was set up by decision of the Council of Ministers to highlight prospects, problems and potential conflicts likely to affect long-term development of the Community and define alternative courses of Community research and development action.

Biotechnology — or more broadly, a "bio-society" scenario — has been selected as one of our three central themes, and within this we shall be sponsoring a number of research studies covering such related long-term issues as Community research and development strategy, manpower and training implications, social acceptability, Third World implications, and environmental engineering; further details are available on request.

I should point out that the FAST programme is distinct from the biomolecular engineering programme described in your issue of 10 January, though naturally we have the benefit of collaboration with our Commission colleagues, both from this programme and elsewhere in the Commission.

Yours faithfully,

MARK F. CANTLEY

Commission of the European Communities, Brussels

Shortage of science teachers

SIR, — It is reported in *Nature* (17 January, page 233) that there are serious shortages of teachers of mathematics and natural sciences, particularly physics, and it is revealed that of our secondary school teachers of physics only 43% are qualified to teach physics. Your report contains the wise and timely warning, "without well-qualified science teachers, there is little hope for the future of science and engineering in Britain". I wish to present the case that the situation is in fact even more serious than the figures suggest.

Firstly, I question the suitability of the education of many (or probably, most) of those who *are* "qualified to teach physics". University graduates generally have acquired an imperfect knowledge of advanced work in a specialist subject. To teach physics in school one requires a very thorough understanding of the elementary principles of the science, and also considerable knowledge of the relationship between physics and other subjects.

Secondly, there is a tacit assumption in all educational thinking that teachers and pupils can easily obtain trustworthy information about any subject by reading textbooks. It is now 20 years since the writer first drew attention to the incredible accuracy of very many physics textbooks, particularly at sixth form level. During the past 20 years a very few publishers have attempted to improve their own books, but the general standard has continued to decline.

As it is obvious that we cannot quickly remedy the shortage of suitably trained teachers, surely it is obvious that we must help the teachers we have by ensuring that they have accurate sources of information.

Yours faithfully,

J.W. WARREN

Brunel University, Uxbridge, Middlesex, UK

Brazil's fuel technology

SIR, — I was very interested in the article on alternative fuel technology in Brazil (6 December, page 550) which you published recently. Unfortunately, in the discussion of ethanol production, the author did not answer the one question which seems to me the most important in this whole project. Where does the fuel come from to distil the ethanol? By fermentation, ethanol can only be produced in dilute aqueous solution, and the amount of energy needed to fractionally distil this may be greater than the energy obtained from burning the ethanol.

It seems to me that this need for an energy input to fractionate the ethanol may be a fatal weakness in the whole ethanol project. It is avoided by the use of methanol, which is not produced in dilute solution, and may be made, for example, from wood *via* charcoal.

Yours faithfully,

DAVID A.H. TAYLOR

University of Natal, Durban, Natal

Nuclear power

SIR, — There seems to have been a serious misprint in the penultimate paragraph of the article on *Nuclear Power: the threat to personal freedom* in the 20-27 December issue (page 774). The paragraph states that the pursuit of nuclear power could conceivably damage civil liberties less than would the pursuit of a high conservation/low energy strategy as outlined earlier this year by the International Institute for Environment and Development. Since the IIED strategy does not touch personal liberties and at most calls for mandatory targets to be set for the energy performance of products such as cars and new buildings, thus merely altering the range of consumer choice in one respect, the paragraph is clearly back to front. Energy conservation may have to be forced through by 'controls' on individuals only if we do 'too little, too late'; but no critic has yet accused the IIED scenario of that! And even controls of this kind are a long way from armed police and security vetting.

Yours faithfully,

GERALD LEACH

International Institute for Environment & Development, London, UK

Job-swapping

SIR, — In regard to your editorial on job-swapping (4 October, page 327), I had the good fortune to exchange jobs and houses with a librarian in Scotland for an academic year and would do it again eagerly.

It did, however, require a fairly substantial amount for travel expenses and salaries, which therefore were paid by our home institutions. I think your point about the necessity for a 3-5 year tenure is unnecessary and, on the contrary, for family reasons, I would suggest a 2-3 year exchange.

Yours faithfully,

DANA L. ROTH

California Institute of Technology, Pasadena, US

New Jersey Turnpike

SIR, — Robert Claiborne (20/27 December, page 776) says that my "attitude to facts is not beyond criticism", but his basis for this statement is manufactured out of context. I said that on a specific occasion the odors and visual obscuration ("like twilight or an eclipse of the sun") described in the book did not occur. The book implied (p102) that this alleged nuisance, caused by the large petrochemical complex near the New Jersey Turnpike, is not even occasionally abated. I lived near the complex for 21 years, and the statement in the book is incorrect.

Yours faithfully,

THOMAS H. JUKES

University of California, Berkeley, US

NEWS AND VIEWS

Physics at very high pressures with laser-driven shock waves

from M.H. Key

RECENT papers by Trainor *et al.* (*Phys. Rev. Lett.* **42,** 1154; 1979) and by Veeser *et al.* (*Appl. Phys. Lett.* **35,** 761; 1979) highlight the fact that in relatively simple experiments laser-driven shock waves can be used to study the propagation of shocks in solids for shock pressures up to terapascals (1 TPa = 10^7 bar = 10^{13} dyne cm^{-2}). In view of the fact that the only demonstrated alternative for studying such high pressure shock waves requires a nuclear explosion (Ragan *et al. JAP* **48,** 2860; 1977) the simplification provided by the laser approach is considerable.

Laser irradiation of a solid surface in vacuum creates a high pressure surface plasma. The pressure exerted on the solid by this 'ablation plasma' is shown in Fig. 1 as a function of laser irradiance I and for two laser wavelengths $\lambda = 0.53$ and 1.05μm. The ablation pressure acts as a 'piston' driving a shock wave into the undisturbed solid. Accurate shock velocity measurements were obtained by Trainor *et al.* and Veeser *et al.* by recording the differential time of transit of the shock through ~30 μm thick foil targets with a step variation in thickness as in Fig. 2. The arrival of the shock at the rear surface was signalled by a fast (~10^{-11} s) rise in luminosity detected by an electron optical streak camera with time resolution ~10^{-11} s.

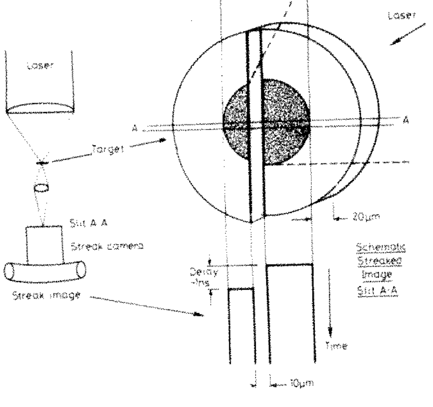

Fig. 2 Schematic diagram showing how streak photography is used to measure shock velocity in a planar foil target with a thickness discontinuity.

The maximum shock pressure for which these techniques are useful is limited by the saturation in the rate of increase of ablation pressure with intensity (Fig. 1) and the associated increasing depth and magnitude of target preheating due to energetic electrons generated in the process of absorption of the laser light in the surface plasma. Where pressure due to this preheating becomes significant relative to the ablation pressure and the preheat penetration depth becomes comparable with the target thickness the process no longer results in a simple shock propagating in an undisturbed medium. Investigation has shown that the preheating depth is a function of $I\lambda^2$ and that the maximum value of $I\lambda^2$ for simple shock generation in these targets is of the order of 3×10^{14} W cm^{-2} μm^2 (Hares *et al. Phys. Rev. Lett.* **42,** 1216; 1979). Thus shock pressures up to 1.8 TPa were reported by Trainor *et al.* using 1.06 μm radiation while Fig. 1 suggests that with $\lambda = 0.53$ μm pressures up to about 10 TPa should be attainable.

The motivation for these studies is that the equation of state of compressed solids is not well known in the interval between a few tenths and a few tens of terapascals. At lower pressures extensive experimental data from static high pressure and chemical explosive experiments are available while at higher pressures the relatively simple Thomas Fermi (TF) theoretical model is valid (Ragan *et al. op. cit.*). The TF model neglects all atomic structure and quantum mechanical binding force between atoms and treats only the compressibility of a degenerate electron gas in the field of positive nuclei. At the opposite extreme a solid at zero pressure is in equilibrium between the degenerate electron repulsive forces and interatomic binding forces. Theoretical analysis for conditions where the applied pressure is not large relative to the binding forces is complex and experimental studies are needed.

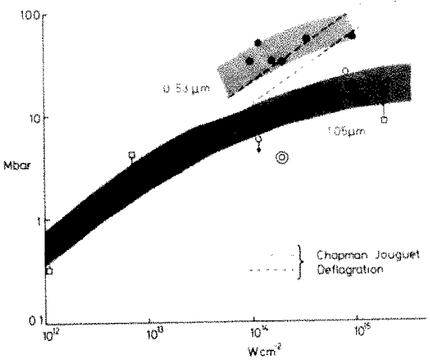

Fig. 1 Ablation pressure in units of megabar as a function of laser irradiance for 0.53 and 1.05 μm laser wavelength. (Dashed lines theoretical model). Unpublished results obtained by research groups from Imperial College London and Queen's University, Belfast working at the SRC Central Laser Facility, Rutherford Laboratory.

The laser technique of shock generation extends the upper pressure limit for such measurements since measurement of any two of the five shock parameters (material density, pressure, internal energy, flow velocity and shock front velocity) allows solution for the other three (from the conservation of energy momentum and mass flow across the shock front) and hence gives the equation of state relationships between pressure, density and internal energy (Ragan *et al. op. cit.*).

M.H. Key is at the Rutherford Laboratory.

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Intermediate filament cycles

from Brian Anderton

RESEARCH into the structure and organisation of the distinct fibrous organelles which make up the cytoskeleton of eukaryotic cells has intensified enormously in the past four or five years. The latest component of the cytoskeleton to come under close scrutiny is the intermediate filament. Intermediate filaments are approximately 10 nm in diameter and are so called because their width lies between those of the other two cytoskeletal fibres, microtubules (24 nm) and microfilaments (6 nm). Unlike tubulin and actin, which are the principal structural proteins of microtubules and microfilaments respectively, intermediate filament proteins seem not to be highly conserved and show a certain degree of tissue specificity. They have been divided into subclasses on the basis of biochemical and immunochemical data and with the inevitable consequence of isolation, the constituent proteins have been given names (in some cases more than one).

Five subclasses have been identified and new ones may need to be added. So far the (roughly tissue-specific) subclasses comprise (1) prekeratin tonofilaments of epithelial cells; (2) desmin or skeletin filaments of smooth muscle which are also identified with the protein present in the z-line region of cardiac and skeletal muscle; (3) filaments of fibroblasts and other mesenchymal cells (vimentin or decamin); (4) neurofilaments of neurones; and (5) glial filaments (glial fibrillary acidic protein) present in astrocytes (but not other glial cells). Structural homologies between different intermediate filaments have only occasionally been sought but some do seem to exist (Gard *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 3894; 1979; Steinert *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 6098; 1978).

The biochemical characterisation of different intermediate filaments has been hampered by the fact that when isolated they are mostly insoluble in salt solutions. This unfortunate physical behaviour from the point of view of the biochemist now seems to have been overcome by Zackroff and Goldman (*Proc. natn. Acad. Sci. U.S.A.* **76**, 6226; 1979). Goldman and his colleagues have for some time been studying the fibroblast intermediate filaments from cells of the baby hamster kidney line BHK-21 and have shown that the main constituents are two polypeptides with chain weights of 54,000 and 55,000 which they have called decamin. In their latest paper they report a detailed investigation of an earlier observation that BHK-21 intermediate filaments disassemble on prolonged exposure to solutions of low ionic strength at around neutral *p*H and reassemble on returning the protein to physiological ionic strength. When disassembled the material seems to consist of short rods or protofilaments perhaps 50 nm long and about half the width of an intermediate filament; the authors make no estimate as to the number of polypeptides constituting a single protofilament.

Turbidimetric measurements during reassembly show the process to be biphasic. Examination by electron microscopy suggests initial lateral association of the short thin rods to produce short thick rods followed by elongation of the latter to give apparently reconstituted intermediate filaments. The assembly process exhibits a critical protein concentration for initiation in the range 0.05–0.15 mg ml^{-1}. Possibly the most significant aspect of the work is the discovery that cycles of disassembly/reassembly can be achieved, although protein recovery seems problematical, and that after two such cycles the stoichiometry of the 54K and 55K polypeptides seems to remain around unity and several high molecular weight proteins above 250,000 copurify with the decamin polypeptides. This approach of inducing cycles of polymerisation/depolymerisation has been used successfully for purifying tubulin and identifying microtubule-associated proteins which remain in a constant weight ratio to the tubulin through repeated cycles. No doubt this report from Goldman's laboratory will now encourage others to apply the same technique in attempts to define the molecular composition of intermediate filaments from different sources. It should be pointed out, however, that Zackroff and Goldman believe the large decamin-associated polypeptides not to be essential for intermediate filament assembly since their removal by mild proteolysis does not block polymerisation of the 54K and 55K decamin polypeptides.

It remains to be seen whether or not the BHK-21 fibroblast intermediate filaments are heteropolymers of the 54K and 55K chains or if homopolymers of each exist separately inside the cell. Lazarides and colleagues have suggested that muscle intermediate filament protein, which they call desmin, is not restricted to muscle in its distribution but is also found in non-muscle cells such as fibroblasts, including BHK-21 cells (*Proc. natn. Acad. Sci. U.S.A.* **76**, 3894; 1979). They have assigned the 54K polypeptide from BHK-21 cells to desmin and the 55K polypeptide to the true fibroblast intermediate filament protein. They have also recently claimed that fibroblast intermediate filament protein too is more widely distributed than hitherto assumed and that it occurs along with desmin in the myofibril z-disc (*Cell* **18**, 1053; 1979). Notwithstanding earlier reports that desmin was absent from non-muscle cells, these more recent data seem convincing and are analogous to the results from Franke's, Weber's and Osborn's laboratories which show that epithelial cells also contain two types of intermediate filament, namely prekeratin tonofilaments and fibroblast intermediate filaments for which they use the name vimentin filaments (*Differentiation* **14**, 35; 1979). However, more studies are needed as the BHK-21 cell line may not be typical. It is unclear, for instance, whether mouse 3T3 cells contain two types of intermediate filament polypeptide as published electrophoretic patterns (Franke *et al. op. cit.*) of purified intermediate filament protein from these latter cells show a single prominent polypeptide and not a doublet like that found from the BHK-21 cells.

Although such ambiguities remain, the classes and distribution of intermediate filaments are now beginning to fall into a pattern although we remain mostly in the dark as to their function. Probably the predominant view is that they are important in maintaining cell shape and the spatial organisation of other intracellular structures. However, now that they promise to be amenable to biochemical investigations of the type used by Zackroff and Goldman, their lack of established function is unlikely to deter the protein biochemists. □

Brian Anderton is a lecturer in the Department of Immunology, St. George's Hospital Medical School, London.

Kimberlite and carbonatite magmas

from K. G. Cox

THE Earth's crust is a relatively thin layer made up of familiar rocks similar to those seen at the surface. However, at a depth of about 10 km in oceanic areas and at 50 km beneath the continents a sudden increase in the velocity of seismic waves marks a fundamental change of structure. From here downwards, to the outer edge of the core at a depth of about 3,000 km, the rocks are referred to as the mantle. This is volumetrically by far the largest compositional component of the Earth but relatively little of a direct nature is known about it. The mantle 50 km below our feet is distinctly less accessible than the Moon. Apart from the very uppermost levels of the mantle, which are occasionally exposed at the surface as a result of violent continental collisions, the only samples available are brought to the surface as fragments ('nodules') suspended in magma during volcanic activity. The distinctly rare volcanoes known as kimberlite pipes, which of course also supply diamonds, are the richest source of such materials.

At a recent meeting in London* J.B. Dawson (University of Sheffield) gave an authoritative review of the present state of knowledge of upper mantle rocks derived as nodules from kimberlite pipes, largely from southern Africa.

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*The Anniversary Meeting of the Mineralogical Society, held in London on 10 January focused on the petrology of mantle materials and deep-seated magmas such as kimberlite and carbonatite.

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The most abundant nodule type is a very magnesium-rich silicate rock termed garnet peridotite and consisting largely of the minerals olivine and enstatite with smaller amounts of garnet and diopside. A distinction can be made between peridotites which are 'fertile' as opposed to 'barren', by which is meant their capacity to give rise to basaltic silicate liquids, the most common surface manifestations of volcanic activity. Barren peridotites are depleted in the low melting components calcium and aluminium, poor in garnet and diopside as a consequence, and seem therefore already to have undergone melting episodes to give rise to basalts. Most of the upper mantle sampled by kimberlites seems to be of this type and has evidently been involved in the melting processes of the past by which the crust has become separated from the mantle.

A distinction is also made between sheared and unsheared peridotite nodules. The former provide evidence of deformational events within the mantle, and it was postulated some years ago that they represented samples of the asthenosphere, that is the supposed layer of mobile mantle underlying the Earth's rigid surface plates. However plate motions as measured by rates of continental drift seem to be far too slow to account for the very strong deformational fabrics observed, and current opinion is drifting towards an alternative theory that the deformation is purely local and connected with the uprise of the kimberlite magma itself.

The study of nodules is the most direct way of obtaining information about the mantle. However many volcanic rocks are the solidified products of melts which originated in the mantle, and so provide another important approach to mantle problems. As well as kimberlite the extraordinary igneous rocks known as carbonatites are an important source of information. These are unique in that they solidify from carbonate, not silicate, melts. The session on kimberlite and carbonatite magmas tackled first the question of whether the two were related. Kimberlites occasionally contain a good deal of magmatic carbonate, both sorts of magmas are emplaced as relatively small intrusions in continental areas and occasionally show a fairly close relation in space and time and both are characterised by concentrations of relatively rare elements. R.H. Mitchell (Lakehead University, Ontario) maintained that because spinel mineral compositions in kimberlites and carbonatites are different the idea of a petrogenetic relation between the two rock types is not supported. S.E. Haggerty and B.M. McMahon (University of Massachusetts, Amherst) disagree, concentrating on mineralogical evidence suggesting that magmatic calcium carbonate was an integral part of kimberlite. Clearly at this stage a definition of what is meant by the term carbonatite is required. Does for example the carbonate component within kimberlite count as carbonatite, or is it simply carbonate? However the lack of definition on this topic is probably less important than the failure to define what is meant by the term petrogenetic relationship. The relationship could consist of a common source material, a common magmatic precursor, a common thermal or deformational event, the exploitation of a common structural weakness in the crust, or some combination of these. W.J. Verwoerd (University of Stellenbosch, S. Africa) in describing the remarkable sequences of aligned intrusions in Angola, S.W. Africa/Nambia and the western part of Cape Province suggested perhaps wisely in view of the complexity of the question, that possibly the only common factor relating the kimberlites and carbonatites in this area was their exploitation of the same structural features. In areas where kimberlites and carbonatites are less common, for example in south east Australia as reviewed by J. Ferguson (Bureau of Mineral Resources, Canberra) and in N. America as reviewed by H.O.A. Meyer (Purdue University, Indiana) any relationship between the two rock types is difficult to find. However the rather close spatial and time relationship between the Murfreesboro kimberlite and the Magnet Cove carbonatite in Arkansas remains an intriguing coincidence.

Concerning the generation of kimberlite magmas D.K. Bailey (University of Reading) produced an ingenious and stimulating hypothesis. He pointed out that continental geotherms (distribution of temperature with depth) deduced from kimberlite nodules from different localities were all closely similar and that these could lie in 'grazing coincidence' with vapour-saturated solidi (onset of melting curves) of mantle rocks. Thus the introduction of fluids (such as water) under such conditions could initiate melting in a very restricted depth range and give rise to kimberlite magmas. Such magmas can only reach the surface if they erupt very rapidly since any attempt to migrate slowly upwards while losing heat results in their solidification. If the thermal gradient is lower than the critical value no melting occurs. On the other hand if it is higher, melting starts at a lower level and the magmas can migrate upwards without immediate solidification, interact with overlying mantle and lose their kimberlitic character. Bailey thus proposes that "kimberlite activity is the limiting case of cratonic magmatism".

On the subject of carbonatites I.C. Freestone and D.L. Hamilton (University of Manchester) reported successful experiments demonstrating immiscibility between natural soda-rich silicate liquids (phonolite and nephelinite) and natural alkali carbonatite. M.J. Le Bas (University of Leicester) also stressed the importance of alkalis in carbonatites pointing out that deduced compositions of fenitising fluids (fluids which transform wall-rocks) and observed compositions of fluid inclusions in calcic carbonates agreed in indicating a significant alkali-metal content.

Much presented at the meeting gave food for thought but probably nothing more so than the account by A.R. Woolley (British Museum) of his re-mapping of the Alnö carbonatite complex. Brogger's work on Fen and von Eckermann's on Alnö are the historical foundations of carbonatite geology, and yet von Eckermann seems to have been wrong on many important points. However, wrong though von Eckermann may have been, he did recognise many important phenomena and that the carbonates were igneous not sedimentary, so Alnö played its part in the development of the science notwithstanding. □

K.G. Cox is in the Department of Geology and Mineralogy, University of Oxford.

Antigravity?

from P.C.W. Davies

NEUTRALISING the effects of gravity has long been a preoccupation among Tibetan monks, yogis, UFOlogists and science fiction writers. On the other hand, Einstein's theory of relativity — the best available theoretical description of gravity — leaves little room for antigravity. This beautiful theory, however, is believed by many physicists to be only an approximation, the reason being that it is inconsistent with the twentieth century's most powerful physical idea — quantum mechanics.

Attempts to unite mathematically Einstein's gravity with quantum mechanics have been frustrated for decades by daunting technical and conceptual problems. Recently, though, a promising new approach has emerged. Known as supergravity, the new theory attempts to treat the familiar gravitational field as only a component part of a more elaborate network of forces and fields. It is within this wider network that hitherto unsuspected forces could arise to neutralise gravity.

Quantum mechanical theory is a set of principles that governs the subatomic world and supergravitators think of gravity in terms of subatomic particles. The ordinary force that keeps our feet on the ground is interpreted in this theory as due to a continual exchange of a hypothetical quantum particle called the graviton. The graviton is gravity's counterpart to the photon of electromagnetism. A crucial difference between gravitons and photons is that the former has twice as much spin as the latter, which can be shown to account for the fact that like electric charges repel, whereas like massive bodies attract. In units of photon spin, the graviton has spin 2.

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For comparison, the elusive neutrino has spin ½, as do electrons and protons, whereas pi-mesons have no spin. The amount of spin carried by a subatomic particle is crucial to the type of mathematics used to describe it. Particles like mesons, photons and gravitons, with integral units of spin, enjoy certain geometrical symmetries that are different for the half-integral spin particles. For many years physicists regarded these two categories of particle as quite distinct forms of matter and energy. Then, in the 1960s, when the variety of subatomic particle species grew bewilderingly fast, it was discovered that many particles hitherto regarded as different could, in a certain mathematical sense, be envisaged as simply different faces of the same basic unit. Physicists began to group the particles into families: the neutron and the proton, for example, became part of an octet of particles that can be thought of as eight slightly different manifestations of just one particle.

In the early schemes, the integral and half-integral spin particles were always kept in separate families because of their very different mathematical symmetries. But in the 1970s several people proposed arrangements in which even those two groups could be mixed together in still larger families, enjoying a new, all-embracing, 'supergravity'. It is from this development that supergravity has arisen. Early models of supergravity put the spin 2 graviton in a small family unit with a completely new particle — the more hypothetical gravitino — having spin 3/2. Soon this model was extended to a family with one spin 2 graviton, two spin 3/2 particles and a single spin 1 particle, like the photon. It is this latter entity that could be responsible for antigravity.

Writing in a recent issue of *Physics Letters* (**88B**, 265; 1979) J. Scherk of the Laboratoire Normale Superieure in Paris, examines the feasibility of the antigravity idea within the context of an extended supergravity model. Basing his analysis on earlier work by K. Zachos of Caltech (*Phys. Lett.* **76B**, 329; 1978), Scherk points out that circumstances could arise in which the spin 1 component of supergravity exactly neutralises the spin 2 gravity. At first sight, the theory seems to predict that the gravitational force between neutrons should be exactly neutralised by spin 1 antigravity. This would render the theory nonsensical — more than half the mass of the Earth is in the form of neutrons. However, the way in which gravity and antigravity affect the matter might be different. Closer examination shows that while ordinary gravity responds to the entire mass of a body, for example a whole neutron, antigravity sees only the elementary constituents. Particles such as neutrons and protons are believed to be composites of three so-called quarks.

Paradoxically, the assembly of three quarks does not have the same mass as three individual quarks. This is because enormous energies are released when the quarks bind together, and this energy also has mass. Taking this into account, Scherk estimates that the effects of antigravity would suppress the Earth's gravitational force on a body by about one part in a million, depending on the proportion of neutrons to protons in the substance concerned. This is well above the experimental upper limit obtained by comparing the rate of fall of differently constituted bodies.

To rescue antigravity from the problems of its own strength, Scherk speculates that although the antigraviton is described by the theory as massless, implying that it travels at the speed of light, it could acquire a small mass spontaneously as a result of a mechanism called symmetry breaking. The upshot of this idea is that the antigraviton is slowed up and the antiforce becomes short in range — perhaps extending for only a couple of metres. It is also greatly depleted in strength. Though this mechanism thereby renders antigravity utterly insignificant under most circumstances, Scherk remarks somewhat tongue-in-cheek that at a high enough temperature the symmetry breaking would fail, and antigravity could be important. The snag is, the temperature concerned would have to be well in excess of billions of degrees. So much for levitation! □

P. C. W. Davies is a lecturer in the Department of Mathematics, King's College London.

The closest cosmic radio source

from Alan Johnstone

VERY few of the radio sources discovered by radioastronomers will ever be visited by spacecraft carrying instruments capable of observing directly the electromagnetic fields and charged-particle distributions which generate the radio waves. Jupiter and Saturn are likely to be the only cosmic radio sources, observable from the ground, where *in situ* plasma measurements can be made. If some spacecraft were able to observe a celestial radio transmitter from within, the information would be extremely valuable, first because it would considerably improve the chances of understanding the source mechanism and second because the resulting knowledge could then be applied to more remote situations where there is no chance of *in situ* measurements.

Fortunately there is no need to send a spacecraft all the way to Jupiter to make these measurements; the closest such radio source is only a few thousand kilometres above the Earth's surface, where radio noise in the kilometric wavelength range (100 kHz — 1 MHz) is generated. Unlike most of the radio noise created in the Earth's magnetosphere it escapes outward from the source region into space because the frequency is above the local plasma frequency. It cannot be detected on the Earth's surface because the frequency is below the peak plasma frequency in the ionosphere, so that all the radiation which starts downward is reflected back out again. The power radiated from the Earth is extremely variable, averaging 10^7 W and reaching peaks in excess of 10^9 W, making it comparable with Jupiter as a radio source. At its maximum the radiated power is 1% of the rate at which energy is being dissipated in the atmosphere by precipitating electrons during an auroral display. This comparison is important because the radiation seems to come from the region where auroral electrons are accelerated and to occur only at times when there are active, discrete, auroral forms visible in the auroral oval.

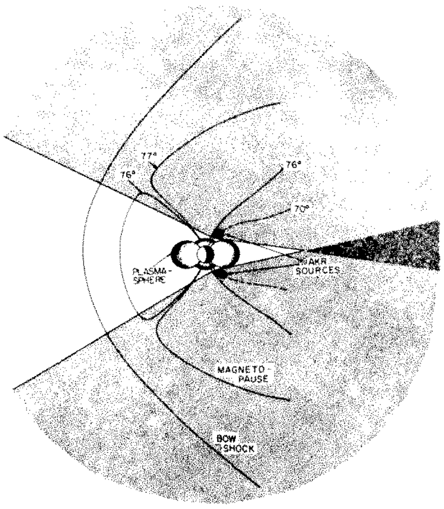

Fig. 1 The time-averaged extent of the conelike emission of auroral kilometric radiation superimposed on a magnetic field model. On the average both sources are observable in the equatorial plane on the nightside beyond 12 R_E (Gallagher & Gurnett *op. cit.*).

It is difficult to locate the source accurately from a spacecraft since an appropriate antenna consists typically of a single, long electric dipole which does not provide sufficient directional information. So far, two methods have been used. The first used the fact that the spin-modulation of the signal strength is a minimum when the antenna is pointed towards the source (Gurnett *J. geophys. Res* **79**, 4227; 1974). The minimum had an angular width of 6° when the source was observed looking back toward the Earth from a distance of 32 Earth radii (32 R_E). This placed the source close to Earth, but did not locate it precisely. The second method used the occultation of the source by the Moon as seen from a spacecraft in orbit around the

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Alan Johnstone is in the Department of Physics, University College, London.

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Moon (Alexander & Kaiser *J. geophys. Res.* **81**, 5948; 1976). They found that the source is strongest and most common in the local time sector from 21 hours to 24 hours, the time at which auroral arcs are most common. From the Moon at first quarter the night-time magnetic field lines are seen in profile. The source of the auroral kilometric radiation then seems to lie along high-latitude field lines within a few R_E of the Earth's surface.

Gallagher and Gurnett (*J. geophys. Res.* **84**, 6501; 1979) have used a totally different approach. They collected data from two satellites for a total of 7 years and found the average intensity of the kilometic radiation as a function of radial distance, local time and magnetic latitude. Despite the variability of the source strength, they find that the average intensities are meaningful in tracing the source. The average intensity integrated over geocentric spherical shells (to find the total outward flux) is negligible below 2.5 R_E, rises to maximum at 4 R_E and then remains essentially level to 30R_E. This places the source between 1.5 R_E and 3 R_E above the surface. The radiation originates at auroral latitudes, between 22 and 24 h local time, spread out over a wide cone (Fig. 1). The average intensity decreases outwards from the source as $1/R^2$ if it is assumed to be at an altitude between 1 R_E and 2 R_E.

This is now known to be where auroral electrons are accelerated by several thousand volts, possibly by an electric field parallel to the magnetic lines of force. There can now be little doubt that the kilometric radiation is related either to the acceleration process itself or to the interaction between the beam-like electron velocity distributions and the cooler ionospheric plasma. Since the radiated power is as high as 1% of the energy of the accelerated electrons, the mechanism — whatever it is — must be a coherent and efficient process. There is no generally-accepted theoretical explanation and a great deal of effort is being expended to find one. There have only been a few *in situ* measurements in the region of interest, despite its relative accessibility, although the number should soon be increased by the NASA Dynamics Explorer Satellites. There should be advances on this scientific front before long.

It is clear that it would have been very difficult to have deduced the type of electron velocity distributions occurring in the source region from the characteristics of the radiated spectrum alone. This emphasises the importance of *in situ* measurements in unravelling the working of complex plasma processes. Many important and common plasma processes occur nearby in the magnetosphere where such measurements can be made easily. Auroral kilometric radiation is similar in spectral and temporal structure to the decametric radiation from Jupiter and the hectometric radiation from Saturn (Kaiser & Stone *Science* **189**, 285; 1975) so lessons learned in the magnetosphere can be applied to Jupiter and Saturn and then perhaps extended to more remote sources.

A new look at the Sherborne bone

from Ann Sieveking

In a recent number of *Antiquity*, (**53**, 211; 1979), R.A.H. Farrar has put forward a case for reconsidering the status of the Sherborne bone. This small engraving of a horse's head and forequarters was published by Arthur Smith Woodward in 1914, as apparently Palaeolithic in date but, 10 years later, was dismissed by Professor Sollas in the third edition of his book *Ancient Hunters and their Modern Representatives* as a forgery (see *Nature* **117**, 86, 233; 1926). The controversy continued in *Nature*, with Sollas emerging as the victor, although more by Smith Woodward's default than by the production of any conclusive proof. The question has been re-examined from time to time, at least with regard to the alleged evidence of hoaxers, or hoaxed, but little attention has been given to the bone itself, except for an analysis of its degree of fossilisation, made by K. Oakley in 1957. Some of the protagonists in fact, apparently including Sollas himself, never looked at the original.

There is little evidence to support a renewed claim for authenticity however. Such evidence as we have about the discovery of this engraved bone is hearsay, contradictory and unacceptable; its provenance, whether it was found in a heap of quarry stones, or in a rubbish heap beside the Bristol road, is without context and its intrinsic characteristics lend little credibility to a Palaeolithic attribution. At this stage the only useful contribution one may make to the problem is in examining the bone itself and here it must be emphasised that it is not the antiquity of the bone that is in question, (Oakley's analysis confirmed it as being fossilised) but the age of the engraving on it.

Smith Woodward maintained that the surface of fossilised bone would flake when engraved and that the Sherborne horse, cut with a relatively clean line, must therefore have been engraved when the bone was fresh. However, tests made by M.H. Newcomer at the Institute of Archaeology do not support this claim: as clear a line may be cut with a flint burin on fossilised bone as on fresh. Further analysis of the technicalities of the engraving (for which observations I am grateful to Dr Newcomer) demonstrates that the surface of the Sherborne bone shows no preparation before engraving, although this is a common feature on Palaeolithic pieces. Under magnification it can be seen that, at certain points, where the engraved line crosses a natural crack in the bone, the cut dips down into such a crack whereas a line engraved before any cracking occurred would maintain an even depth. In addition, the engraved lines of the head are discontinous and hesitant which, although in no way conclusive is, again, uncharacteristic of Palaeolithic work. Lastly, in the region of the mane of the Sherborne horse, the original surface of the bone has flaked away in antiquity, leaving a rougher surface below. The engraved lines of the mane, however, start on the smooth original surface of the bone and continue onto the rougher flaked surface (See Figs 1 and 2) at the edge. Had the engraving been contemporary with the fresh bone, these lines could be expected to have been truncated by the flaking, and the fact that they are not suggests that the engraving has been added subsequently to an already degraded bone.

Finally the stylistic characteristics of the Sherborne engraving must be considered. Farrar remarks that the Sherborne horse has an upstanding mane and lacks clearly defined ears, both of which characteristics may be paralleled in Palaeolithic examples, for instance in the Creswell Crags engraving (in the British Museum collections) and that less characteristically the Sherborne horse shows no linear definition below the mane and has an unusually drooping muzzle. He cites a parallel to this atypical muzzle in an illustration in Graziosi (*Palaeolithic Art*, 1960; Pl. 134.b); this illustration, however, is a drawing and not a photograph of the original engraving in the cave of Pindal and in this respect of the drooping muzzle it is inaccurate. The engravings of horse heads from La Grande Paroisse, Pincevent and from Cépoy in Loiret, which are closer than is Pindal in date, location and the form of support used to the English Upper Palaeolithic art pieces, show an animal with a short, broad

Fig. 1 The Sherborne bone (actual size ~8.5 cm), now in the collections of the British Museum (Natural History). Photo: Jill Cook.

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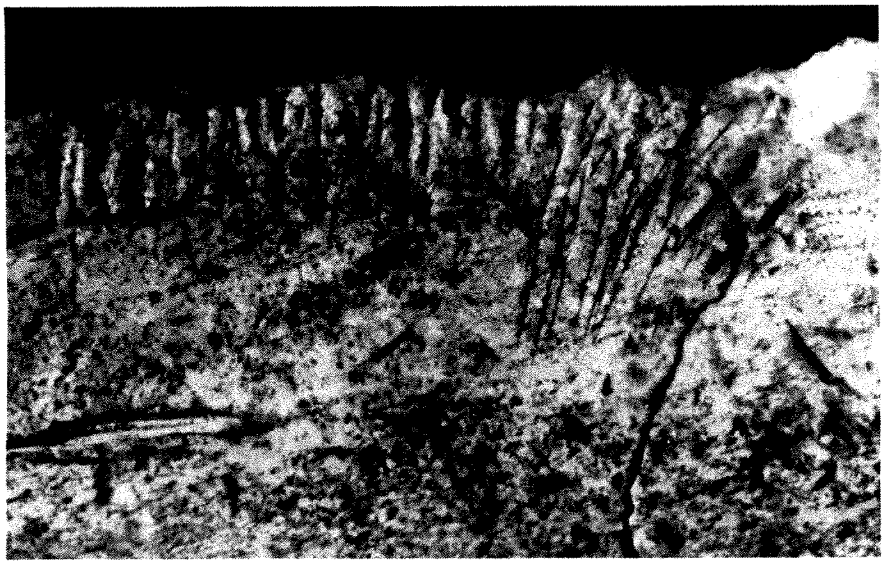

Fig. 2 Enlargement of the mane of the Sherborne horse. Photo: Jill Cook.

head that is very similar to that engraved on the Creswell Crags bone and rather dissimilar to the Sherborne variant. Sollas suggested that the illustration of the Creswell Crags horse, published (for the second time) by Boyd Dawkins in 1880, was the inspiration for the Sherborne piece, in that both show head and forequarters of a horse, facing right, with an upstanding mane. There seems some force in this argument, for the Sherborne horse is much more similar to Boyd Dawkins' rather poor illustration than to the original Creswell piece. Side by side the Sherborne and Creswell originals have little in common, the Creswell horse, for example is extremely delicately engraved, but this is not a characteristic that is conveyed in the illustrations and it is not a characteristic repeated in the Sherborne engraving. Furthermore, if the Sherborne copyist had seen the original Creswell engraving he might have appreciated the importance of the numerous additional odd lines that occur on the surface of this bone. Some of these appear in Boyd Dawkins' illustration, but might here well be dismissed as cracks, although they are a very consistent feature of Palaeolithic decoration. It is unusual to find a Palaeolithic engraving that is devoid, as is the Sherborne horse, of any extraneous lines, though these are not always included in illustrations of the material. Obviously there is no way of conclusively determining the age of an engraving as opposed to the age of its bone support, but such evidence as can be cited suggests that the Sherbone horse is not an authentic Palaeolithic engraving. The catalogue of Palaeolithic art pieces from this country is very small but to augment it by including the Sherborne horse appears to be quite unjustifiable. □

Ann Sieveking researches into Palaeolithic Art at the Institute of Archaeology, London.

Geology and health

from a Correspondent

THE influence of minerals and chemicals naturally occurring in the environment on health, together with the problems that arise in the mining and industrial use of minerals were reviewed at a recent meeting in London*. P. C. Elmes and J. C. Wagner (MRC Pneumoconiosis Unit) reviewed the diseases resulting from occupational and environmental exposure to mineral dusts. On the one hand are those which lead to lung scarring and malfunction such as silicosis, coal miners' pneumoconiosis and asbestosis, in which the severity of the symptoms is directly related to the intensity and length of exposure, and on the other are the more insidious cancers, where exposure may be small and the time lag to distress and diagnosis as much as 30–40 years. The load of dust in the lung associated with disease varies widely, from several hundred grams of coal dust, 5-10 g of pure silica to as little as 1 mg of asbestos. Both asbestos exposure and smoking increase the risk of lung cancer, but combining the two multiplies the risk. A recent discovery of an extremely high prevalence of the rare pleural mesothelioma in the village of Karain, in Cappadocia, Turkey, where in one year all 11 deaths out of 800 inhabitants were due to this one disease (there were no cases in the surrounding villages), has been linked with the presence of a zeolite mineral, erionite, which is found in the volcanic tuff rock from which local houses are built, and which weathers to form the soil on which this agricultural community depends. Similar fibrous minerals of 0.01–0.5 μm in diameter and 5–15 μm in length, a dimension that is retained in the body tissues and causes cell damage, occur in other geological materials and must be a cause of concern.

The large-scale development of natural resources to meet the energy crisis will have important environmental consequences. W. Chappell (Center for Environmental Sciences, University of Colorado) forecast the production of 1 million barrels of oil a day from oil shale in the US by the late 1990s, requiring the processing of 500 million tons of shale per annum, as much as the present-day coal production in the US. Problems of waste disposal, release of toxic trace elements and organics into surface and ground waters and emissions of SO_2 into the atmosphere will expose workers and local communities to possible health hazards. Projected large scale expansion in surface coal mining over the next five years has led to studies linking epidemiology and water quality in the Midwestern USA, a programme funded by the Environmental Protection Agency and undertaken by Betsy Kagey (Downstate Medical Center, New York) and B.G. Wixson and N.L. Gale of the Environmental Research Center, University of Missouri — Rolla.

Geochemical investigations linking heavy metals in soils and the prevalence of dental caries were described by B.E. Davies (University of Wales, Aberystwyth) and R.J. Anderson (Dental School, University of Birmingham). Caries incidence has been found to be above normal where soils are heavily contaminated with lead from old metalliferous mining and smelting activities.

The Applied Geochemistry Research Group at Imperial College and the Institute of Geological Sciences have over the years mapped most of the country and geochemical atlases of England and Wales, Northern Ireland and parts of Scotland have been published. Research initiated by John Webb in the early 1960s has shown that regional geochemical differences can be successfully related to the health of crops and livestock, and that maps have uses in agriculture, water resources, pollution studies and public health. The Atlases will continue to provide unique sources of data on many different elements which can aid in selecting areas for food, water and medical surveys, particularly in rural parts of the country.

J. Lag (University of Oslo) reported that the Academies of Science in the five Scandinavian countries are setting up a committee to organise further cooperation in geomedical investigations. This will in part fulfil a similar role to the Working Party on Environmental Geochemistry and Health in Britain established in 1979 by the Royal Society under the chairmanship of S.H.U. Bowie. Consultation and cooperation in this expanding multidisciplinary research area should now be possible between scientists in the UK, Scandinavia, USA, Canada, USSR and Australia which now all have similar groups of interested scientists. □

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*A one-day discussion meeting on Geology and Health was held on 30 January at the Geological Society of London, Burlington House, and organised by Dr Iain Thornton, Imperial College.

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REVIEW ARTICLE

A list of interstellar molecules

A. P. C. Mann & D. A. Williams
Mathematics Department, UMIST, Manchester M60 1QD, UK

Interstellar molecules are useful guides to the conditions within interstellar clouds. The list presented here gives information on the sources, abundances and conditions of all interstellar molecules identified up until September 1979.

THE number of molecules identified in the interstellar medium has increased dramatically during the past decade: Fig. 1 shows the number discovered per year. As well as being of intrinsic interest, the molecules are useful tracers of the interstellar gas, and they can inform us of conditions within the interstellar clouds. The wide chemical variety present interesting problems of formation.

We present in Table 1 a list of interstellar molecules presently (September 1979) identified, and some information pertaining to them. The information selected is that likely to be of interest to a general reader and to those interested in the physics and chemistry of interstellar clouds, rather than in making observations. The first discovery is noted, and survey references are given, with some information regarding sources, abundances and conditions. An excellent compilation of radio and microwave frequencies of observed interstellar molecular transitions has been prepared by Lovas, Snyder and Johnson[1]. We do not attempt to duplicate that information here.

For convenience, we have attempted to make Table 1 as concise as possible and have not included all lines observed for each molecule. In general, the information for each molecule is usually given on two lines of the table: the information on each line is from the reference on that line. The first line is devoted to the discovery reference, the second to a survey reference, if available. (Some particularly important molecules—H_2, OH and CO—are given extra space.) A description of Table 1 now follows; the molecules are arranged by number of atoms they contain, and then by molecular weight.

Column 1

Chemical formula, and name: the main isotopic version is given first; minor isotopes follow, in order of atomic weight. Atoms not identified by an isotropic weight are the predominant isotope: that is, C implies ^{12}C.

Column 2

References: the first reference given is that of the first observation; the information on the same line in Table 1 refers to this reference. On the second line of information for the same molecule, another reference is given, where possible. This is a reference to a survey, if available.

Column 3

Sources. On the first line are given the sources in which molecule was first observed; on the second line the number of sources in the survey reference, or main sources in the second reference.

Column 4

Spectral line: this contains some information about the spectral lines observed. Ultraviolet (UV), optical (OPT), and infrared electronic (IR) transitions are indicated. Otherwise, the lines are in the microwave or radio spectral regions. The type of transition is briefly indicated; electronic bands defined; 'Rot' implies a rotational transition; Λ implies that Λ-doubling is observed; and 'Hfs' implies that hyperfine structure is detected in the observations.

Column 5

Spectrum: absorption (A), emission (E), or maser emission (M) is indicated.

Column 6

Column density: the number given is the column density, per cm^2, calculated by the authors of the work referenced, under the assumptions listed in **column** 7. If column densities are not available, other relevant information, such as abundance ratios, may be given.

Column 7

Conditions: assumed (or inferred). Assumed information may involve the total gas number density n, per cm^3, and temperature (such as rotation, spin, excitation, and kinetic) in degrees Kelvin. The type of calculation used by the authors is also mentioned. Inferred information about the source, if given, is in brackets.

Column 8

Comment: any useful remark is given here.

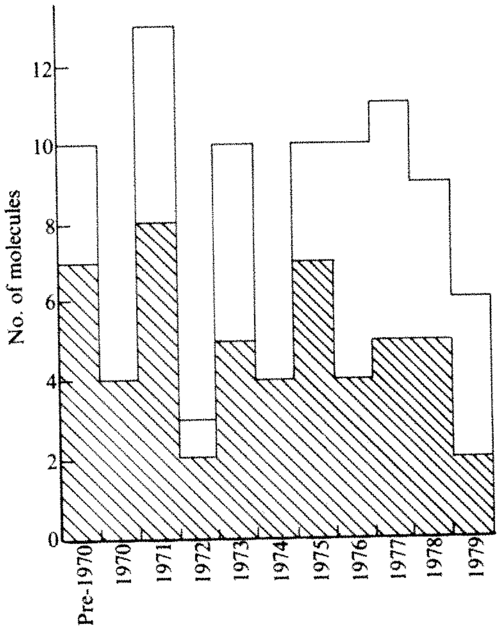

Fig. 1 Rate of discovery of interstellar molecules in recent years. Hatched totals ignore isotopic varieties.

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Table 1 Interstellar molecules

Column 1 Molecule	2 Refs	3 Sources	4 Spectral line	5 Spectrum	6 Column density	7 Conditions assumed (or inferred)	8 Comment
Diatomics							
H_2	6	ξ Per	UV: Lyman bands	A	$\sim 10^{20}$	$(T_{10} = 60)$	a, Detected where $E(B-V) \geq 0.03$
Molecular hydrogen	7	a	UV: Lyman bands	A	b	$(T_{10} = 77 \pm 17)$	b, $[H_2] \approx [H]$ where $E(B-V) \geq 0.1$
	8	BN, KL	IR 1–0 Vib–Rot	E	$\sim 10^{19}$	$T \sim 2000$	see also refs 9, 10
HD	11	9, inc. ξ Per	UV: Lyman bands	A	10^{14}–10^{15}	T_{10} from	HD formed and destroyed more
Deuterium hydride	12	13 sources	UV: Lyman bands	A	$< 10^{15}$	H_2 observations	rapidly than H_2
CH^+	13	75 stars	OPT: A–X bands	A	$\leq 10^{13}$	opt. thin	
Methylidyne ion	14	300 stars	OPT: A–X bands	A			see also ref. 15
$^{13}CH^+$	16	ζ Oph	OPT: A–X bands	A	$\sim \frac{1}{80}[CH^+]$	opt. thin	Negative results for $^{13}CH^+$ at
	—						ref. 17; ref. 16 single line only
CH	18	Many: a	OPT: $^2\Delta$–$^2\Pi$ bands	A	$\leq 10^{13}$	—	a, seen in 25% of sample 300
							OB stars, see ref. 14
Methylidyne	19, 162	Cas A	$^2\Pi_{1/2}, J = \frac{1}{2}$, Λ, Hfs	E	b	Non thermal	b, Surveys (refs, 21, 22) $N_{\text{radio}} \leq 10^{14}$
OH	23	Cas A	$^2\Pi_{3/2}\ J = \frac{3}{2}$, Λ, Hfs	A	$\sim 2 \times 10^{14}$	LTE, $T = 10$	1st MW emission lines, see ref. 24
Hydroxyl	26	Survey, 87 sources		M	—	—	Many other MW lines detected
	27	oPer	UV: D–X	A	$\sim 10^{13}$		Tentative; other UV observed in ref. 28
^{17}OH	25	Sgr A	$^2\Pi_{3/2}\ J = \frac{3}{2}$, Λ, Hfs	A	a	opt. thin	a, $[^{18}OH] \approx 4[^{17}OH]$
^{18}OH	29	Sgr A	$^2\Pi_{3/2}\ J = \frac{3}{2}$, Λ, Hfs	A	$\sim 10^{12}$	opt. thin	Agrees with terrestrial ratio
	30, 31	Sgr A, B2	$^2\Pi_{3/2}\ J = \frac{3}{2}$, Λ, Hfs	A		opt. thin	$[^{16}O]/[^{18}O] > 200$
C_2	32	Cyg OB2 No. 12	IR Phillips bands	A	$\sim 10^{14}$	$T_X \approx 35$	
Diatomic carbon	33	ζ Oph	IR Phillips bands	A	$\sim 8 \times 10^{12}$	$T = 22$	
	34	ζ Oph	UV: D–X(0–0)R(0)	A	3×10^{12}	$T_R \leq 16$	4σ detection
CN	18	Many: a	OPT: B–X bands	A			a: see in 25% of sample 300
Cyanogen	35	Ori A, W51	1–0, Rot, Hfs	E	$\sim 10^{15}$	Boltzmann, $T_R = 50$	OB stars, see ref. 14 Survey ref. 36
CO	37	Ori A + 8 others	1–0, Rot	E	$> 10^{17}$	See ref. 38	V. abundant, found in V. many sources
Carbon monoxide	39, 40	Surveys	1–0, Rot	E	10^{18}–10^{19}	$T = 10$–50	(2–1) Rot, see ref. 41; (3–2) Rot, see ref. 42
	43	ζ Oph	UV: A–X Bands	A	10^{15}		see also ref. 44
	45	3 sources	IR: 1–0 Vib Rot	A	$\sim 10^{20}$	$T = 650$	Circumstellar. other bands see ref. 34
^{13}CO	43	ζ Oph	UV A–X Bands	A	$\approx 10^{-2}$ [CO]	CO opt. thick	See also refs 39 and 46
	47	W51; Sgr A, B2	1–0, Rot	E			See ref. 45 for Vib–Rot lines
$C^{17}O$	20	Ori A, ρ Oph	1–0, Rot	E	$[C^{18}O]/[C^{17}O]$	opt. thin	
	48	9 sources	1–0, Rot	E	≈ 4	opt. thin	
$C^{18}O$	47	W51; Sgr A, B2	1–0, Rot	E		opt. thin	
	39	8 sources	1–0, Rot	E		opt. thin	
$^{13}C^{18}O$	39	NGC2024	1–0, Rot	E	v. weak	opt. thin	
NO	49	Sgr B2	$^2\Pi_{1/2}, J = \frac{1}{2} \leftarrow \frac{3}{2}$, Λ	E	2×10^{16}	Thermal, $T = 20$	Also assumed $N = 10^{24}$
Nitric oxide	—					opt. thin	Apparently very rare in Galaxy
CS	50	Ori A, +3 others	3–2, Rot	E	10^{13}–10^{15}	opt. thin thermal	
Carbon monosulphide	51	30 sources	1–0,2–1, Rot	E	10^{13}–10^{15}	$T = 40$, $n = 10^5$	Only observed in dense regions
^{13}CS	50	Ori A, +3 others	3–2, Rot	E		as CS	Deduced: $[^{12}C]/[^{13}C] \sim 90$, (40 at Sgr)
	52	Sgr B2 + 3 others	1–2–3, Rot	E			
$C^{33}S$	51	Sgr B2	2–1, Rot, Hfs	E		opt. thin	Triplet splitting seen
	52	Sgr B, Ori A	3–2, Rot, Hfs	E	$C^{33}S/C^{34}S$		
$C^{34}S$	51	Ori A, +4 others	2–1, Rot	E	≈ 0.2	opt. thin	
	52	Sgr B, +3 others	3–2, Rot	E			$[^{13}CS]/[C^{34}S] \approx 0.4$–$0.6$
SiO	53	Sgr B2	3–2, Rot, $v'' = 0$	E	$\sim 4 \times 10^{13}$	Thermal $T = 50$	Usually in maser emission near
Silicon monoxide	54	16 sources	1–0, Rot, $v'' = 1$	E, (M)		opt. thin	stars and H II regions. See also ref. 55
^{29}SiO	56	Ori A	2–1, Rot, $v'' = 0$	E	~ 0.05 [SiO]	opt. thin	Deduces SiO not masering in
	—						Ori A
^{30}SiO	57	Ori A, Sgr B2	2–1, Rot, $v'' = 0$	E		opt. thin	Not masering
	—						
SO	58	Ori A + 6 others	4_3–3_2–2_1, Rot	E	$\leq 2 \times 10^{14}$	See ref. 69	
Sulphur monoxide	59	18 sources	5 Rot transitions	E	$\leq 2 \times 10^{14}$		Kleinman–Low Neb., $N(SO) \sim 10^{16}$
^{34}SO	59	Ori A, KL	3_2–2_1, 2_3–1_2, Rot	E	a	opt. thin	a, consistent with terrestrial
	—						$^{34}S/^{32}S$
NS	60	Sgr B2	$^2\Pi_{1/2} J = \frac{3}{2} \leftarrow \frac{5}{2}$, Λ, Hfs	E	$\sim 10^{14}$	$T = 20$, $n = 10^4$	
Nitrogen sulphide	61	Sgr B2	$^2\Pi_{1/2}\ J = \frac{3}{2} \leftarrow \frac{5}{2}$, Λ, Hfs	E	?	Stat. eqm.	Anomalous excitation?
SiS	62	Sgr B2, IRC + 10216	6–5–4 Rot	E	4×10^{13}	$T = 50$. opt. thin	Sgr B2: 6–5 only. Tentative?
Silicon sulphide	—						
Triatomics							
H_2O	63	Sgr B2, +2 others	6_{16}–5_{23} Rot	E, M	$> 10^{17}$	?	High excitation (465 cm^{-1} above ground)
Water vapour	64, 161	9, 10 sources	6_{16}–5_{23} Rot	M			Surveys: all maser sources
HDO	65	Ori A, KL	1_{10}–1_{11}, Rot	E, M	10^{16}–10^{17}	$T = 70$, $n = 10^7$	Not as strongly masering as H_2O
	—						
$H_2{}^{18}O$	66	Ori A, KL, DR21	3_{20}–3_{13} (para), Rot	E, M	$\sim 4 \times 10^{-3}\ cm^{-3}$	$T \sim 50$, $n = 10^6$	Weak maser. Assuming terrestrial $^{16}O/^{18}O$, then $n(H_2O) = 8\ cm^{-3}$
	—						
C_2H	67	13 sources	1–0, Rot, Hfs	E	5×10^{14} -3×10^{15}	$T = 100$	Upper limit: levels thermalised Lower limit: lowest two levels only
Ethynyl	—						
HCN	68	6 sources	1–0, Rot, Hfs	E	$\sim 10^{15}$	Boltzmann, $T = 20$	
Hydrogen cyanide	69	4 sources	1–0, Rot, Hfs	E	a		a, extensive modelling of line width, n_T, T
DCN	70	Ori A	2–1–0, Rot, Hfs	E	$\sim 4 \times 10^{12}$	Thermal, $T = 10$	a, DCN/HCN usually high
	71	7 sources	2–1, Rot, Hfs	E	a		(lower in Sgr B2)
$H^{13}CN$	68	Ori A, Sgr B2	1–0, Rot, Hfs	E	$[DCN]/[H^{13}CN]$ variable		
	71	14 sources	1–0, Rot, Hfs	E			Other refs: 73 and 69
$HC^{15}N$	72	Ori A	2–1, Rot	E		opt. thin	$[HC^{15}N]/[H^{13}CN] \approx 2.6$
	73	6 sources					Another survey in ref. 74
HNC	75		1–0, Rot	E	T_X uncertain		ref. 76 is lab. confirmation of microwave spectrum
Hydrogen isocyanide	77	OriA, +3 others					
DNC	77	OriA, +3 others	2–1, Rot	E	$[HNC]/[DNC]$	a	a, abundance of DNC higher in
	78	NGC2264	1–0, Rot	E	variable		cooler clouds?
$HN^{13}C$	79	5 sources	1–0, Rot	E			
	—						
$H^{15}NC$	80	DR(21)OH	1–0, Rot	E	$[H^{15}NC]$ $\sim [HN^{13}C]$	opt. thin	Enrichment of ^{15}N(?)
	—						
HCO^+	81		1–0, Rot	E	—	a	Predicted: (ref. 82); lab. confirmation: ref. 83
Formyl ion	84, 85	Surveys					a, theory predicts HCO^+ most abundant ion

Table 1—continued

Column 1	2	3	4	5	6	7	8
Molecule	Refs	Sources	Spectral line	Spectrum	Column density	Conditions assumed (or inferred)	Comment
DCO^+	86	3 sources	1–0, Rot	*E*	$[H^{13}CO^+] \sim [DCO^+]$	opt. thin	High $[DCO^+]/[HCO^+]$ implies $X(e) < 10^{-8}$ in dense clouds
	87	8 sources					
$H^{13}CO^+$	85	10 sources	1–0, Rot	*E*	$\lesssim 0.1[HCO^+]$	opt. thin	Confirms HCO^+ detection See also ref. 86
	—						
$HC^{18}O^+$	89	L134, TMC1	1–0, Rot	*E*		opt. thin	$[H^{13}CO^+]/[HC^{18}O^+] \sim 6$
	90	Sgr B2	1–0, Rot	*E*		opt. thin	~terrestrial isotope ratios
HCO	91	4 sources	$1_{01}–0_{00}$, $J = \frac{3}{2} – \frac{1}{2}$, $F = 2–1$	*E*	$\sim 10^{13}$	$T = 20$	$[HCO] < [HCO^+]$
Formyl	—						
N_2H^+	92	11 sources	1–0, Rot, Hfs	*E*	$\sim 3 \times 10^{13}$	$T_s = 10$	ref. 93: calculation, and ref. 94: lab. spectrum
	—						
N_2D^+	95	L134N	1–0, Rot, Hfs	*E*	$\sim 10^{12}$	opt. thin. $T_K \approx 15$	$N_2H^+/N_2D^+ \approx 2$. Enormous isotope fractionation
	—						
H_2S	96	7 sources	$1_{10}–1_{01}$ Rot	*E*	$\lesssim 10^{15}$	opt. thin $T = 20$	
Hydrogen sulphide	—						
HNO	97	Sgr B2, NGC2024	$1_{01}–0_{00}$, Rot	*E*	$\lesssim 10^{15}$	opt. thin	$N \lesssim 10^{14}$ (NGC2024)
Nitroxyl	—				(Sgr B2)	Boltzmann $T < 100$	tentative?
OCS	98	Sgr B2	9–8 Rot	*E*	$\lesssim 3 \times 10^{15}$	opt. thin	
Carbonyl sulphide	99		Several, Rot	*E*		Boltzmann $T < 50$	See also ref. 100
SO_2	101	Ori A, Sgr B2	Several, Rot	*E*	$\sim 10^{16}$	opt. thin	Narrow (interstellar?) and broad (circumstellar?) components seen
Sulphur dioxide	—					Boltzmann $T \approx 100$	
4-atomics							
NH_3	102	Sgr A	(1,1)(2,2) inversion	*E*	$\sim 2 \times 10^{16}$	opt. thin	Seen in many dense clouds, possibly 'clumpy'?
Ammonia	106	11 sources	(1,1) (2,2) inversion	*E*			
NH_2D	103	Sgr B2	$1_{11}–1_{01}$, Rot	*E*	$\sim 10^{14}$	$n \sim 10^6$, opt. thin	$[NH_2D]/[NH_3] \sim 0.017$
	104	Orion, KL	$1_{11}–1_{01}$, Rot	*E*	$\sim 10^{14}$	$50 \lesssim T_K \leqslant 150$	
$^{15}NH_3$	105	Orion	(1,1)(2,2)(3,3)	*E*	a	opt. thin $T_R \sim 50$	*a*, $[^{14}N]/[^{15}N] \approx$ terrestrial
	—						
C_2H_2	107	IRC+10216	Several, Rot	*A*	$\lesssim 10^{17}$	LTE $T = 300$	Circumstellar source, not 'genuine' interstellar
Acetylene	—						
H_2CO	108	17 sources	$1_{11}–1_{10}$, Rot	*A*	$\sim 10^{14}$(Sgr A)	a	a, $1_{10}–1_{10}$ is seen in absorption against 2.7 K BB
Formaldehyde	110	28 sources		*A*			
HDCO	109	L134N, CLD2	$2_{02}–1_{01}$, Rot	*E*	a	$T_{Kin} = 10$	$[HDCO]/[H_2CO?] \lesssim 0.1$ D enhanced
	—	—					
$H_2{}^{13}CO$	111	Sgr A, B2, W51	$1_{11}–1_{10}$, Rot	*A*	a	opt. thin	a, $[H_2CO]/[H_2{}^{13}CO] \sim 10$ in galactic centre. W51 tentative
	112	Sgr B2					
$H_2C^{18}O$	113	Sgr B2	$1_{11}–1_{10}$, Rot	*A*	$(^{13}C:{}^{12}C)/(^{18}O:{}^{16}O) \approx 10$	opt. thin	Using ref. 111
	114	Sgr A, B2, W33	$1_{11}–1_{10}$, Rot	*A*	b	opt. thin	b, $[H_2{}^{13}CO]/[H_2C^{18}O] \approx 7$
HNCO	116	Sgr B2, W51	$4_{04}–3_{03}$, Rot	*E*	$\sim 5 \times 10^{14}$	opt. thin	Also $1_{01}–0_{00}$ detected, ref. 115
Isocyanic acid	—						First report ref. 75
H_2CS	117	Sgr B2	$2_{12}–2_{14}$, Rot	*E*	$> 10^{16}$	opt. thin	See also ref. 59
Thioformaldehyde	118	Sgr B2	$4_{14}–4_{13}$, Rot	*A*	?		Non thermal
C_3N	119	IRC+10216	10–9–8, Rot	*E*	$\lesssim 10^{15}$		Based on calculated spectrum, ref. 120
Cyanoethynyl	—						
HNCS	163	Sgr B2	Rot, 3 lines	*E*	$\sim 2 \times 10^{13}$	$T_R = 14 \pm 5$, opt. thin	
Isothiocyanic acid		—					
	—	—					
5-atomics							
CH_4	122	Ori A	6 lines, Rot	*E*	$\sim 10^{22}$(?)	Non thermal excitation	Column density uncertain
Methane	—						Tentative identification
CH_2NH	123	Sgr B2	$1_{11}–1_{10}$ (Mult) Rot	*E*	$> 3 \times 10^{14}$	opt. thin. $T_x \gg 25$	
Methanimine	—						
CH_2CO	124	Sgr B2	Several, Rot	*E*	10^{14}	Stat. eqm.	Tentative identification of 3 more Rot. lines
Ketene	—					$n > 10^6$, $T_k \gtrsim 20$	
NH_2CN	125	Sgr B2	$4_{13}–3_{12}$, $5_{14}–4_{13}$, Rot	*E*	2×10^{13}	Stat. eqm.	
Cyanamide	—				-6×10^{14}	$n \lesssim 10^6$, $T_k \lesssim 60$	
HCOOH	126	Sgr B2	$1_{11}–1_{10}$, Rot	*E*	$10^{12}–10^{13}$		Tentative? See ref. 5
Formic acid	127	Sgr B2	$2_{11}–2_{12}$, Rot	*E*	$\sim 5 \times 10^{13}$	$T_R \approx 2.2$	
C_4H	128	IRC+10216	12–11–10–9–8, Rot	*E*	$4 \times 10^{14}–3 \times 10^{15}$	$60 < T_{ex} < 600$	Detection based on theoretical calculation of rotational spectrum
Butadiynyl	—						
HC_3N	129	Sgr B2	0–1 Rot	*E*	a: $\sim 10^{14}$	opt. thin. $T = 50$	a, corrected, see ref. 130
Cyanoacetylene	131	17 sources	7 transitions	*E*	$> 10^{14}$	Stat. eqm.	See ref. 131 for other refs
$H^{13}CCCN$	132	Sgr B2	1–0, Rot	*E*	$\sim 0.05[HC_3N]$	opt. thin	
	133	Sgr B2, Ori A	9–8 Rot	*E*			$^{12}C/^{13}C = 22$(Sgr B2), 50(Ori A)
$HC^{13}CCN$	134	Sgr B2	0–1 Rot	*E*			
	133	Sgr B2, Ori A	9–8 Rot	*E*			
$HCC^{13}CN$	134	Sgr B2	0–1 Rot	*E*			
	133	Sgr B2, Ori A	9–8 Rot	*E*			
6-atomics							
CH_3OH	135	SgrA, B2	$1_{10}–1_{11}$ Rot	*E*		opt. thin	Column densities higher towards Sgr and KL nebula
Methyl alcohol	136	14 sources	2–1, several, Rot	*E*	$\sim 6 \times 10^{14}$	Stat eqm.	Other transitions ref. 88
$^{13}CH_3OH$	136	Sgr B2	2–1, several, Rot	*E*		Stat. eqm.	$[^{13}CH_3OH]/[CH_3OH] \approx 0.1$
CH_3OD	136	Sgr B2	2–1, several, Rot	*E*		Stat. eqm.	$[CH_3OD]/[^{13}CH_3OH] \approx 0.1$
CH_3CN	137	Sgr A, B2	6–5 Rot	*E*	2×10^{14}	Stat. eqm.	
Methyl cyanide	138	Ori A	5–4 Rot	*E*		$n > 10^6$, $T > 150$	
NH_2CHO	139	Sgr A, B2	$2_{11}–2_{12}$ Rot, Hfs	*E*	6×10^{14}	Stat. Eqm	
Formamide	140	Sgr A, B2	$1_{11}–1_{10}$, Rot, Hfs	*E*	$\sim 10^{15}$	$T = 100$, $n = 10^5$	
CH_3SH	121	Sgr B2	Rot, 6 lines	*E*	$\sim 10^{14}$	$T_R = 9 \pm 3$, opt. thin	
Methylmercaptan	—	—					

Table 1—continued

Column 1 Molecule	2 Refs	3 Sources	4 Spectral line	5 Spectrum	6 Column density	7 Conditions assumed (or inferred)	8 Comment
7-atomics							
CH_3NH_2	141	Sgr B2, Ori A	5_{15}–5_{05}, 4_{14}–4_{04}, Rot	*E*	$\sim 10^{15}$	Boltzmann	
Methylamine	142	Ori A	2_{02}–1_{10}, Rot	*E*	$\sim 10^{15}$	$T_x = 40$	
CH_3NHD	143	Sgr A, B2 Ori A, W51	$2^{(+)}_{02}$–$1^{(-)}_{10}$ Rot	*E, A*		$30 \leq T_x \leq 60$	Tentative Detection, not confirmed by independent search ref. 144
	—						
CH_3C_2H	145	Sgr B2	5_0–4_0, $k = 1$–4, Rot	*E*	$\sim 3 \times 10^{15}$	opt. thin. Boltzmann	
Methylacetylene	138	Sgr B2	5_0–4_0, $k = 1$–4, Rot	*E*		$T_R = 31$, $n \geq 10^4$	
CH_3CHO	146	Sgr A, B2	1_{10}–1_{11}, Rot	*E*	$> 3 \times 10^{14}$	opt. thin	Maser? (ref. 147)
Acetaldehyde	147	Sgr B2	2_{11}–2_{12}	*E*			See also ref. 138
CH_2CHCN	148	Sgr B2	2_{11}–2_{12} Hfs Rot	*E*	$\sim 4 \times 10^{13}$		Maser
Vinyl cyanide	149	Sgr B2	$10_{0,10}$–$9_{0,9}$, Rot	*E*	$\sim 3 \times 10^{13}$		ref. 150: 2–1 line detection
HC_5N	151	Sgr B2	4–3 Rot	*E*	$\sim 10^{13}$	opt. thin. Boltzmann $T = 30$	Also ref. 152. Weak maser.
Cyanodiacetylene	153	TMC 1, 2	9–8 Rot	*E*	$\sim 10^{14}$		
8-atomics							
$HCOOCH_3$	154	Sgr B2	1_{11}–1_{10} Rot	*E*	$\sim 2 \times 10^{13}$	1% inversion	Tentative
Methyl Formate	—						
9-atomics							
CH_3CH_2OH	155	Sgr B2	Three lines, Rot	*E*	$\sim 10^{15}$	Stat. Eqm. $T = 20$, $n = 10^4$–10^5	
Ethyl alcohol	—						
$(CH_3)_2O$	156	Ori A	6_{06}–5_{15} Rot	*E*	$\sim 10^{15}$	Boltzmann $T = 10$–100	Also: 2_{20}–2_{11}–2_{02} tentatively identified
Dimethylether	—						
CH_3CH_2CN	157	Ori A, Sgr B2	24 lines OriA 7 lines Sgr B2 } Rot	*E*	$\sim 10^{14}$	LTE $T = 20$ opt. thin	
Ethyl cyanide							
HC_7N	158	Heiles 2 ≡ TMC 1	9–8, 21–20, Rot	*E*	$\sim 3 \times 10^{13}$	Stat. Eqm. $T = 8$, $n = 3 \times 10^3$	Similar results assuming LTE
Cyanotriaceylene (or cyanohexatriyne)	159	TMC 1, 2	22–21, Rot	*E*	$\sim 2 \times 10^{12}$		
11-atomics							
HC_9N	160	Heiles 2	18–17, 25–24, Rot	*E*	$\sim 3 \times 10^{12}$	opt. thin $T = 11$, $n = 4 \times 10^4$ Coll. Ex.	Rot. spectrum extrapolated from cyanopolyene series
Cyano-octatetra-yne							

Discussion

We have occasionally indicated that a detection may be only tentative. Factors tending to confirm the presence of a molecule are: (1) the existence of several spectral lines, all showing the same velocity shift; (2) detection in a number of astronomical sources; (3) the identification of an isotopic version of the molecule at the corresponding frequencies; and (4) hyperfine structure at the correct splitting. Thus, for example, HCO^+ observed in but a single line, is confirmed not only by the laboratory spectrum, but also by the isotopic versions $H^{13}CO^+$ and DCO^+, at the correct frequencies, in the same astronomical sources.

Some ambiguity occurs: when is a molecule circumstellar, and when interstellar? Clearly no nice division occurs, and we have not attempted to make one. The source Sgr B2 towards the galactic centre remains the richest, but there are some surprises here; it does not exhibit (as yet) the three most massive molecules known. Isotopic ratios are important indicators of interstellar chemistry, and, again, the galactic centre seems to be an anomalous region in this respect. Fractionation of isotopes is apparently very effective; certain isotopic versions may be overabundant by orders of magnitude.

Of these 90 molecules, the chemistry of, perhaps, 10 is thought to be understood, though theoretical models are often in conflict with observation. The list is a reminder of the complexity of the problems awaiting solution. The interested reader will find the astrophysical context well reviewed in refs 2–5.

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ARTICLES

Interstellar extinction in the Large Magellanic Cloud

K. Nandy*, D. H. Morgan*, A. J. Willis†, R. Wilson†, P. M. Gondhalekar‡ & L. Houziaux§

* Royal Observatory, Blackford Hill, Edinburgh EH9 3HJ, UK; † Department of Physics and Astronomy, University College, London, Gower Street, London WC1E 6BT, UK; ‡ SRC Appleton Laboratory, Ditton Park, Slough, Buckinghamshire, UK; § Institut d'Astrophysique, Université de Liège, Belgium

Recent UV observations together with complementary visible data of several reddened and comparison stars of similar spectral types in the Large Magellanic Cloud have been used to study the interstellar extinction in that galaxy. Most of the reddened stars studied here are located within 2° of 30 Doradus and show remarkably high extinction in the far UV, suggesting a large abundance of small particles. From the optical wavelength to 2,600 Å *the normalised extinction curves of the LMC stars are similar to the mean galactic extinction law.*

THE Large Magellanic Cloud (LMC) is the nearest extragalactic system and a preliminary study of the UV spectra of LMC members shows the importance of the IUE[1] for stellar and interstellar work in the Magellanic Clouds. The Clouds contain dust though to a lesser extent than the Galaxy. A recent survey of the chemical composition of H II regions in the Magellanic Clouds[2] reveals that oxygen is deficient in these galaxies by about a factor of four relative to the Orion nebula. The composition of the interstellar dust and gas in the Clouds provides important information on the origin of the dust and the chemical enrichment of the interstellar media in these galaxies.

The wavelength dependence of interstellar extinction over a wide range of wavelengths from the visible to the UV depends on the composition and relative abundances of the agents responsible for scattering and absorption[3]. The characteristic feature of the galactic extinction curve is the broad symmetric absorption band ($\Delta\lambda \sim 750$ Å) centred near 2,200 Å (ref. 4). Earlier attempts to determine the UV extinction law in the LMC, using surface brightness measurements obtained with the

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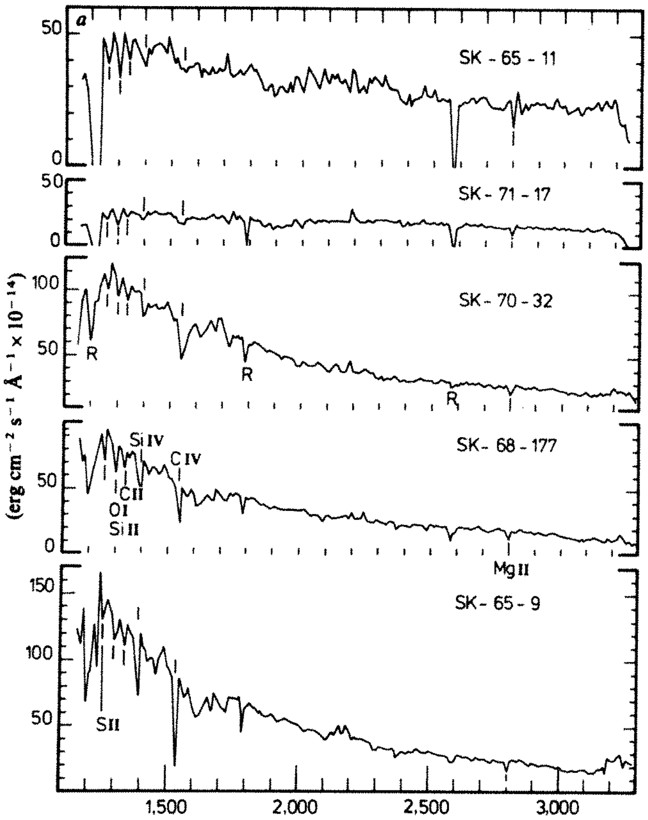

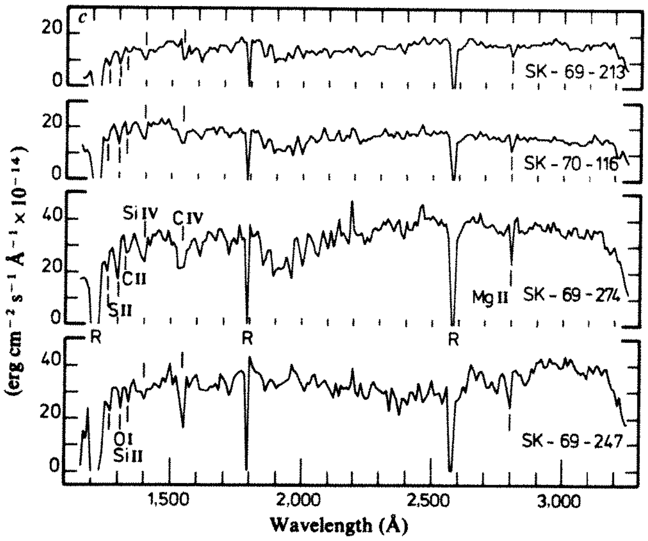

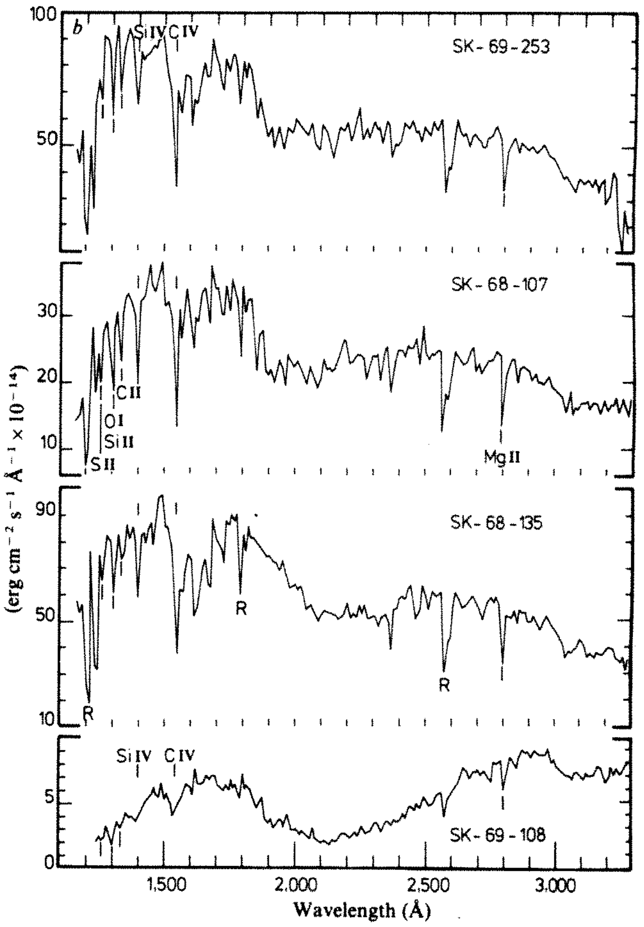

Fig. 1 *a*, The IUE spectra of five comparison LMC stars showing the major interstellar lines (S II, O I, Si II, C II, Mg II), stellar C IV and Si IV lines, and the fiducial marks indicated by R. *b*, *c*, The IUE spectra of eight reddened LMC stars. The lines shown are as *a*.

S2/68 (ref. 5) and ANS experiments[6,7] have led to discrepant results. The detection of the 2,200 Å feature in the spectrum of a reddened LMC member, SK-69-108, has recently been reported[8]. This article presents further observations, in both the visible and the UV, of a larger sample of LMC stars to study interstellar extinction in the LMC.

Observations

The IUE[9,10] controlled from the ESA satellite tracking station at Villafranca, Spain, was used in its low resolution mode ($\Delta\lambda \sim 6$ Å) to obtain UV spectra of the reddened and comparison stars. Complementary visible spectra which extend from 3,980 to 6,140 Å were obtained at a dispersion of 110 Å mm^{-1} using the image dissector scanner at the Cassegrain focus of the 3.6 m telescope of the ESO at La Silla, Chile. Table 1 lists the reddened and comparison stars observed, together with their coordinates, photometric data and spectral types[11–13].

The reduced UV spectra provided before August 1979 by the IUE ESA Observatory contained ITF errors in the short wavelength channel. Consequently, the photometric correction of the raw images of these spectra was repeated with the correct intensity transfer functions (ITF) using a program developed by J. Fettle (personal communication), and the corrected spectra extracted. The UV spectra in the long wavelength channel were provided by the ESA Observatory. Absolute fluxes were obtained using the adopted sensitivity calibration curve[14].

The wavelength scales were checked by examining the positions of the principal interstellar lines: 1,260 Å (blend of Si II and S II), 1,303 Å (O I, Si II), 1,335 Å (C II), 2,342 Å (Fe II), 2,382 Å (Fe II), 2,598 Å (Fe II) and 2,798 Å (Mg II). The wavelength scales of the reduced spectra were found in all cases to be accurate to ±4 Å. The raw images were checked for fiducial marks, noise, spikes and saturation effects. Data affected by saturation and fiducial marks were excluded. Except for the short wavelength spectrum of SK-69-213, all spectra were well exposed with average signal over all spectral ranges of interest lying over 100 DN (IUE data number). The UV flux distributions in absolute units smoothed over 10 Å bands of the comparison and reddened stars are shown in Fig. 1*a–c*.

The visible spectra were obtained on one night (25 December 1978). The wavelength and flat field calibration of the data, recorded on magnetic tape, was performed at ESO, Geneva, using He–Ar and flat field spectra taken immediately before the stellar observations. As the difference between the air masses at the beginning and the end of the observing night was small, the visible spectra were corrected for atmospheric extinction using the mean law appropriate for La Silla (by Dr H. Pederson of the ESO). The visible spectra were reduced to give data points every 1.5 Å and in the subsequent analysis average counts in 60 Å wavelength intervals from $\lambda^{-1} = 1.66$ to $2.54\ \mu\text{m}^{-1}$ were utilised. The photometric accuracy of the average counts is $\sim\pm3\%$.

Measurement of interstellar extinction

The analysis of the spectral features in the UV and visible data obtained will be given elsewhere. Note, however, that the reddened stars listed in Table 1 clearly show the diffuse interstellar feature at 4,430 Å similar to that observed in reddened stars in the galaxy. The equivalent width of this feature is compatible with that computed from the colour-excess, $E(B-V)$, from the mean galactic relationship between W_{4430} and $E(B-V)$ (ref. 15).

To measure the extinction in the visible wavelength range, a set of extinction curves was derived for each of the reddened stars using comparison stars, and a mean extinction curve was determined for each reddened star, thus reducing the errors introduced by possible spectral type mismatch. The extinction in magnitudes, $\Delta m(\lambda)$ has been normalised to $\Delta m = 0$ at 5,500 Å (the effective wavelength of the V-band) and $\Delta m = 1$ at 4,300 Å (the effective wavelength of the B-band). Within observational errors, the individual extinction curves in the visible agree well thereby allowing a mean extinction curve to be constructed. The mean curve was computed in the wavelength range, $\lambda^{-1} = 1.66$–$2.94\ \mu\text{m}^{-1}$ with r.m.s. error $<\pm0.3$ mag, and extended to the U-band using the available UBV photometric data. This mean curve seems to be identical to the mean galactic extinction law in the visible as derived by Seaton[16].

For the measurement of interstellar extinction in the UV, the comparison stars SK-67-108 and SK-67-111, which were observed with the small (3×3 arc s) IUE spectrograph slits, were excluded. To improve the photometric accuracy of the UV data, the stellar fluxes were averaged in 50 Å bands from 1,150 to 3,000 Å. The mean photometric accuracy of a single observation has been estimated from the difference in the colours $(m_\lambda - m_{2{,}900\text{Å}})$ of stars with more than one observation: it is $\sim\pm0.05$ mag except near the extremities of the wavelength channels where it is much larger, about ±0.3 mag.

The UV extinction has been measured for pairs of reddened and comparison stars which match in spectral type. The extinction, Δm is normalised to $\Delta V = 0$ and $\Delta E(B-V) = 1$, where ΔV is the difference in the V magnitudes and $\Delta E(B-V)$ is the difference in the colour-excesses of the reddened and comparison stars. The UV extinction curves for eight reddened stars are shown in Fig. 2; the ordinate is the normalised extinction,

$$\Delta m = \frac{A_\lambda - A_V}{E(B-V)}$$

Discussion

Apart from photometric errors, the major source of error in the extinction measurements constructed from the pairs of reddened and comparison stars is the mismatch in spectral type and luminosity class. For the sample of LMC stars studied here, the spectral type error is estimated to be $\sim\pm1$ subclass. This estimate is based on an examination of the important spectral features both in the visible and in the UV. However, the difference in absolute magnitude, ΔM_V between the reddened and comparison stars is ~-1.6 mag. Galactic supergiants have a flux deficiency in the far UV (shortward of 1,600 Å) as compared with giants and dwarfs[17]. However, from a study of the spectra of galactic stars which appear in the Ultraviolet Bright-Star Catalogue[18] it is found that the UV flux distributions of B0-B3Ia stars do not differ significantly from those of B0-B3Ib stars, the differences being well within the photometric errors. The error in the measured extinction due to spectral type mismatch of ±1 subclasses per unit normalising factor is $\sim\pm0.2$ mag near 2,740 Å increasing to $\sim\pm0.4$ mag at 1,400 Å.

The interesting feature of Fig. 2 is that shortward of $\lambda^{-1} = 3.8\ \mu\text{m}^{-1}$ the extinction curve for SK-69-108 is clearly different from those of the other stars: shortward of $5.5\ \mu\text{m}^{-1}$ the extinction for SK-69-108 is on average 2 mag lower than that for the other stars and near 2,200 Å ($4.6\ \mu\text{m}^{-1}$) it is ~1 mag greater. But longward of 2,600 Å ($\lambda^{-1} = 3.8\ \mu\text{m}^{-1}$), the normalised extinction values for all the stars are the same. Note that the reddened star SK-69-108 is situated in the bar, while the other reddened stars are located to the north-east of the bar within 2° of the 30 Doradus region, a site of recent star formation. This raises the interesting possibility that the extinction properties of the dust in the latter region may differ from those of the dust in

Table 1 Programme stars observed in the LMC

Star	RA (1975)	Dec (1975)	Spectral type	V	$B-V$	$U-B$	Spectral range observed
Reddened stars							
SK-68-107	5h 30.8 min	−68° 26′	B0Ia	12.22	0.03	−0.82	Visible to UV
SK-68-135	5 38.0	−68 56	09.5I	11.36	0.00	−0.86	Visible to UV
SK-69-108	5 20.1	−69 54	B3I	12.10	0.27	−0.49	Visible to UV
SK-69-213	5 36.4	−69 12	B1	11.97	0.10	−0.75	UV
SK-69-253	5 39.7	−69 28	B0I	11.23	−0.02	−0.81	Visible to UV
SK-69-247	5 39.1	−69 31	B6I	10.42	0.17	−0.54	UV
SK-69-274	5 41.7	−69 49	B2.5Ia	11.21	0.04	−0.74	UV
SK-70-116	5 49.3	−70 3	B2Ia	12.05	0.11	−0.72	UV
Comparison stars							
SK-65-9	4 59.2	−65 51	B1	12.72	−0.22	−1.07	UV
SK-65-11	4 59.3	−65 45	B5I	11.66	−0.04	−0.69	UV
SK-67-108*	5 26.5	−67 38	B0	12.56	−0.20	−1.05	Visible to UV
SK-67-110	5 26.9	−67 29	B1:	11.62	−0.07	−0.94	Visible
SK-67-111*	5 26.9	−67 30	0B	12.57	−0.20	−1.03	Visible to UV
SK-68-177	5 53.0	−68 14	B2	12.93	−0.18	−0.96	UV
SK-70-32	5 0.5	−70 13	B0I	13.10	−0.21	−1.01	UV
SK-71-17	5 22.0	−71 58	B3Ia (ref. 13)	12.29	−0.06		UV
SK-69-249	5 39.2	−69 30	B0.5	(10.6)			Visible

* IUE observations recorded using the small spectrograph aperture. All other IUE data were obtained using the large aperture. Figure in brackets is photographic V-mag (ref. 11).

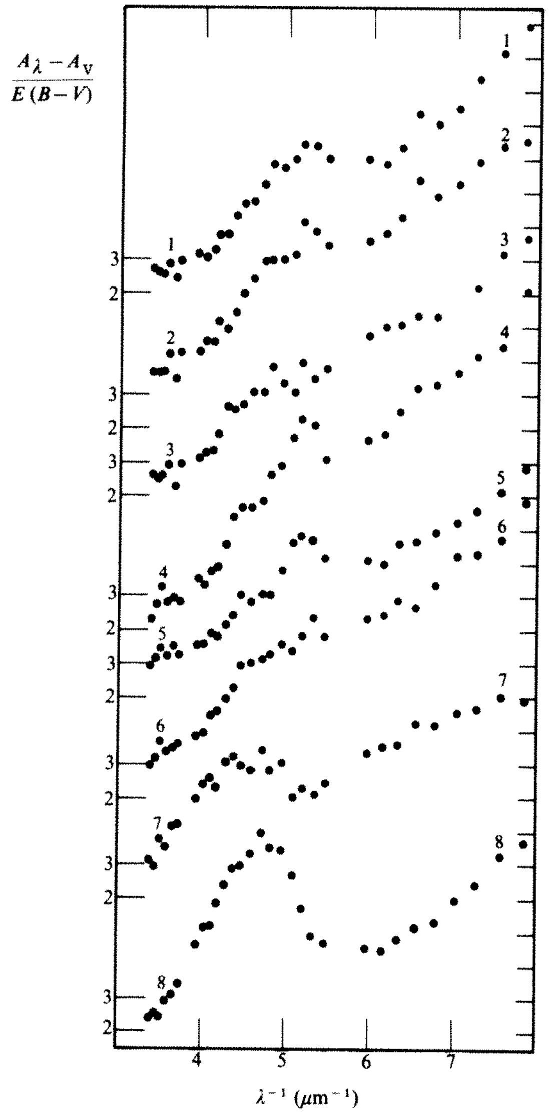

Fig. 2 Individual extinction curves normalised to $V = 0$ and $E(B-V) = 1$ derived from IUE observations. The numbers 1–8 denote the following pairs of reddened and comparison stars: 1, SK-68-107, SK-70-32; 2, SK-69-253, SK-70-32; 3, SK-68-135, SK-70-32; 4, SK-69-274, SK-68-177 and SK-71-17; 5, SK-70-116, SK-68-177; 6, SK-69-213, SK-65-9; 7, SK-69-274, SK-65-11; 8, SK-69-108, SK-71-17.

the bar. As the extinction curves of seven of the stars have similar shapes, they have been combined to give a mean curve. This curve is shown in Fig. 3 with the extinction curve for SK-69-108; also shown in Fig. 3 are the mean visible extinction curve as determined for the LMC stars, and for comparison the galactic extinction law of Seaton[16]. The mean normalising factor being 4, the r.m.s. error bar shown in the mean extinction curve can be explained in terms of the observational errors. Within this error, the extinction curve for SK-69-108 is close to the galactic law, while the mean extinction curve for other stars shows an increase in extinction by ~3 mag in the far UV shortward of $\lambda^{-1} = 6\ \mu\mathrm{m}^{-1}$, which is much larger than twice the r.m.s. error.

The 2,200 Å feature for the mean curve seems to be weaker than that for the galactic law, but not as much as suggested by Koornneef[7]. For example, the colour-excess ratio $E(2{,}200-2{,}500)/E(B-V)$ has a mean value of 2 for the seven stars, (2.7 for SK-69-108) and 2.5 for galactic stars. Between 4.6 and 5.5 $\mu\mathrm{m}^{-1}$ the mean extinction curve does not decrease as much as it does in the case of SK-69-108 and in the galactic law. This is partly due to the broad 1,920 Å feature ($\Delta\lambda \sim 100$ Å) of stellar origin, being much stronger in the spectra of five of the seven reddened stars than in the comparison stars. This 1,920 Å feature is due to a blend of Fe III lines, first detected in the S2/68 spectra of luminous stars and found to be independent of spectral type[19]. The structure near $\lambda^{-1} = 5.2\ \mu\mathrm{m}^{-1}$ in the mean extinction curve is due to this stellar feature. But when corrected by using interpolated continua for the 1,920 Å feature, the extinction near 1,920 Å decreased by only a few tenths of a magnitude. Therefore the difference between the mean extinction curve for the seven stars and the galactic law as well as SK-69-108 seems to be real shortward of 2,200 Å. A possible explanation for the increasing extinction in the far UV for the LMC region around 30 Doradus is that the relative abundance of small (a few hundred ångströms sized) dielectric particles is significantly greater than that in the interstellar medium in the solar neighbourhood.

The difference between the extinction curves for SK-69-108, located in the bar, and the other seven stars may indicate a regional difference in the extinction law in the LMC. Clearly further observations of reddened stars in the LMC bar are needed.

Conclusion

From the study of seven reddened stars located within 2° of 30 Doradus in a region to the north-east of the bar of the LMC, and five comparison stars, the mean extinction curve for this region shortward of 1,600 Å, shows significantly greater extinction than the mean galactic extinction law. This may be due to a relatively higher abundance of small (few hundred ångströms sized) dielectric particles. However, an eighth reddened star, SK-69-108, shows an UV extinction curve similar to the mean galactic law: but this star is located in the bar of the LMC, suggesting a possible regional variation of the far UV extinction law throughout the LMC.

The interstellar extinction curves of all the stars in the visible and near UV wavelength ranges show no significant differences from the mean galactic extinction law.

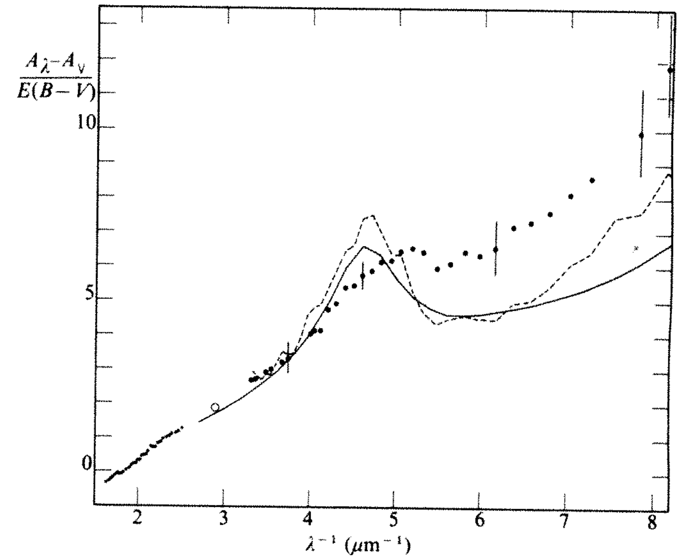

Fig. 3 The mean extinction curve derived from IDS data (dotted line); the mean extinction in the U-band (○); and the mean UV extinction curve for seven stars situated in a region within 2° of 30 Doradus (●). The dashed lines denote the extinction curve for SK-69-108 (situated in the bar). The solid line is the mean interstellar extinction law for the galaxy.

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An extensional origin for the Buchan and Witchground Graben in the North Sea

P. A. F. Christie

Department of Geodesy and Geophysics, Madingley Rise, Madingley Road, Cambridge CB3 0EZ, UK

J. G. Sclater

Department of Earth and Planetary Sciences, MIT, Cambridge, Massachusetts 02139

Seismic refraction and subsidence observations are used to explain the heatflow and subsidence properties of the North Sea.

THE theory of plate tectonics has been very successful in describing the creation of oceanic crust and accounting for the heat flow and subsidence anomalies observed over mid-ocean ridges[1]. However, the theory has not been able to explain the heat flow or subsidence of continental shelves or of epicontinental basins, in particular the Mesozoic and Cenozoic subsidence of the North Sea[2], which is considered here.

The main structural elements of the North Sea are the Viking and Central Graben, which underlie the axis of Tertiary sinking of the North Sea basin. These two major graben meet the Moray Firth Basin in the region of the Forties oil field, which also marks the confluence of the Buchan and Witchground Graben. The North Sea has been a centre of subsidence since the Devonian and although the graben system was initiated in Permo–Triassic times, most of the extension occurred from the Jurassic to Mid-Cretaceous[3]. Major rifting seems to have died out by the start of the Upper Cretaceous, giving way to a broad, general subsidence which allowed the accumulation of chalk, sands from the north-west and finally a thick sequence of Tertiary clays, shales and siltstones.

Although there are many models to account for the formation of shelves and inland basins (see refs 4–6), the most attractive are those based on the concepts used in describing the evolution of lithosphere as it moves away from the mid-ocean ridges[7–9]. In particular, on the basis of a high heat flow and a shallow Moho in the Aegean Sea, McKenzie[8,10] has suggested a simple stretching mechanism to account for the formation of sedimentary basins. In this model, thinning of the lithosphere occurs as a result of large scale extension whereby the surface area is increased by a factor b. If the extension is rapid and lithosphere is conserved, both the crust and the lithosphere decrease in thickness by a factor of $1/b$, causing a fault controlled isostatic subsidence of the surface and creating a thermal anomaly by the passive upwelling of hot asthenospheric material. As the thermal anomaly decays by conduction through the crust, the lithosphere thickens and subsides allowing, in the absence of further extension, the formation of a saucer shaped sedimentary basin. It can be shown[8] that the heat flow and subsidence history of a basin can be predicted as a function of the extension factor. If crustal material is conserved, a measurement of the depth to the Moho will indicate the amount of extension when compared with the Moho depth beneath a non-extended region. We show here that the decrease in Moho depth and increase in subsidence beneath the Witchground and Buchan Graben can be accounted for by a mechanism involving between 50 and 100% stretching of the lithosphere from the Jurassic through to the Mid-Cretaceous.

Seismic refraction data

The Marine Group at Cambridge University undertook the investigation of the velocity structure of the crust and upper mantle along a line parallel to and west of the main axis of Tertiary sinking in the North Sea (Fig. 1). The refraction experiment involved the shooting of three lines into sea bottom seismometers designed and built at Cambridge specifically for this experiment[11]. The main line, reversed over 400 km, was fired across the Witchground and Buchan Graben and was complemented by a shorter crustal control line at each end. Crustal and upper mantle phases were identified from the seismograms and interpretation of the travel time information was made using the method of time term analysis (see ref. 12). Using this approach, each shot and receiver location is assigned a delay time, or time term, which is due to the presence of the overburden between the shot or receiver point and the buried refractor. If the velocity structure in the overburden is known, then the delay time to the refractor beneath a particular site may be inverted to a depth value.

The analysis of the Firth of Forth profile revealed three refractors beneath the sediments with velocities of 5.7, 6.2 and 7.2 km s^{-1}. The Moho velocity is ~8.16 km s^{-1}, a value taken from the Main Line as the Moho refraction was not reliably identified as a first arrival on this profile. The overall depth to the Moho is ~30 km compared with 32–33 km observed for the Moho depth beneath the Southern Uplands as revealed by the LISPB experiment[13]. For the crustal control line at the north, a time term analysis of these arrivals, together with the crustal arrivals from Main Line shots, gave good control over the crustal refractors although the quality of the data was not as high as for the Firth of Forth Line. On the East Shetland Platform, the short range refractions gave a phase velocity of ~4.1 km s^{-1}, which is consistent with Devonian Old Red Sandstone at shallow depth. This overlies an upper crustal layer of 5.7 km s^{-1}, which in turn covers a layer of 6.2 km s^{-1}. Both these values are in good

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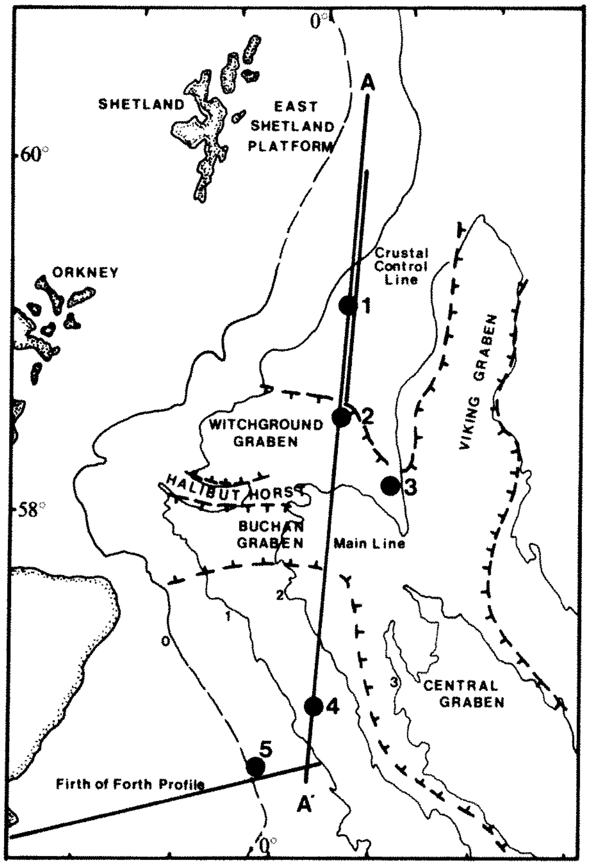

Fig. 1 Seismic refraction lines, drill holes, the major Mesozoic faults and Tertiary isopachs in the Central North Sea. The three refraction lines are the Firth of Forth Line, the Crustal Control Line and the Main Line.

agreement with the velocity structure determined from the Firth of Forth Profile, although difficult correlations make identification of a lower crustal refractor uncertain. On the main north–south line, energetic mantle arrivals were observed on most of the seismograms, but with greatly varying waveform suggesting the superposition of rays. Arrivals were generally well defined and a time term analysis yielded an upper mantle velocity of 8.16 km s^{-1}, which is controlled by good reversed coverage. The delay times to the Moho computed in the analysis increase towards the centre of the line, as does the sediment thickness.

A large number of oil companies have allowed generalised velocity–depth structures to be compiled from their individual well logs. By combining this information with the crustal refraction data already deduced, the Main Line delay times were converted into depths, enabling a seismic section to be constructed (Fig. 2). After stripping off the delay due to the sediments, the Moho has an anticlinal form beneath the sedimentary basin, with the shallowest Moho depth of ~25 km below the deepest sediments. Further north, the Moho deepens again to ~32 km beneath the East Shetlands Platform. The anticlinal structure can cause focusing of the refracted rays to produce the varying waveforms observed. The asymmetric position of the Moho anticline with respect to the centre of the profile can also explain the asymmetric amplitude peaks on the Main Line records, if focusing is admitted. The amplitude peaks are at receiver R3 from the northern shots and receiver R8 from the southern shots[14].

Subsidence data

Basement subsidence as a function of time was estimated in a suite of five holes across the Witchground and Buchan Graben from lithologic and biostratigraphic logs provided by the Shell/Esso, Conoco, Mobil and Amoco oil companies. From these data, the generalised lithologic sections (Fig. 3*a*) were constructed. To calculate the basement depth at a given time, it is necessary to remove the younger sediments, allow for the effects of compaction on the older sediments, compute the isostatic loading effect of the uncompacted sediments and add the water depth at the time of deposition[15,16]. We have ignored the effects of eustatic sea level changes. In the decompaction process, the base of the Upper Permian salt or anhydrite was taken as being basement. As the regression at the end of the Aptian–Albian is an almost basinwide reflective marker at the beginning of the phase of general subsidence, this event has been taken as the datum for plotting the depths of subsequent time–stratigraphic units. This unconformity is evidence that the North Sea was generally close to sea level at the beginning of the Upper Cretaceous, a view which is also supported by reports that shallow-water faunal assemblages have been found in the uppermost Lower Cretaceous[17]. Hence, plots of depth relative to the Aptian–Albian are graphs of approximately true depth below sea level.

In contrast to Clarke[2], who suggested that the subsidence rate was increasing during the Tertiary, we find that the subsidence varies almost linearly with time (Fig. 3*b*). When corrected for the depth of deposition of the sediments, as estimated from faunal assemblages by W. A. Berggren and F. M. Gradstein (personal communcations) and by analysis of the sands at hole 2, the exponential tail off of subsidence rate with time becomes more pronounced. Along the profile the subsidence varies from about 500 m to ~1,300 m, with the greatest amount of subsidence in the centre of the graben which coincides with the shallowest Moho depth.

Discussion

An estimate of the stretching factor can be obtained from the seismic section, assuming that stretching stopped at the base of the Upper Cretaceous and that crustal material has been conserved. The depth from base Upper Cretaceous to the Moho at R5 is ~20 km, while under the East Shetlands Platform (or the Southern Uplands) it is roughly 32 km. This indicates a thinning of about 0.6 or a stretching of 1.6. If the value of 35 km for the depth to the Moho beneath the Shetland Islands is used as the estimate of the thickness of the unstretched part of the lithosphere, a value of 1.7–1.8 is obtained for the stretching factor.

Estimates of the amount of stretching can also be obtained from the amount of post-Lower Cretaceous subsidence. The observed subsidence of the five holes was examined and compared with that predicted by the model for stretching factors of 1.25, 1.5 and 2.0. The subsidence on the flanking blocks of the graben system indicates only sight stretching whereas the subsidence curves within the graben suggest stretching values of between 1.5 and 2.0, which covers the range of values given by the seismic evidence.

The stretching mechanism is supported by the heat flow which is higher than normal[18]. The observed values are obtained from extrapolated Bottom Hole Temperatures and estimates of thermal conductivities, and could well be too high. Reducing these values by only 10% gives a value that is close to that expected if 35 km of continental crust with a mean radiogenic component of heat flux of 0.8 μcal cm^{-2} s^{-1} is stretched by a factor of between 1.5 and 2.0 (Table 1).

Another observation accounted for by this mechanism is the 2–3 km of Middle Jurassic to Lower Cretaceous sediment observed within the graben itself. The initial subsidence is probably dominantly fault controlled and occurred during the extensional process. It is caused by isostatic adjustment as a result of the replacement of light crust by denser asthenospheric material. Given the amount of extension deduced from the seismic and subsidence data, the amount of this initial fault controlled subsidence within the graben may be computed from

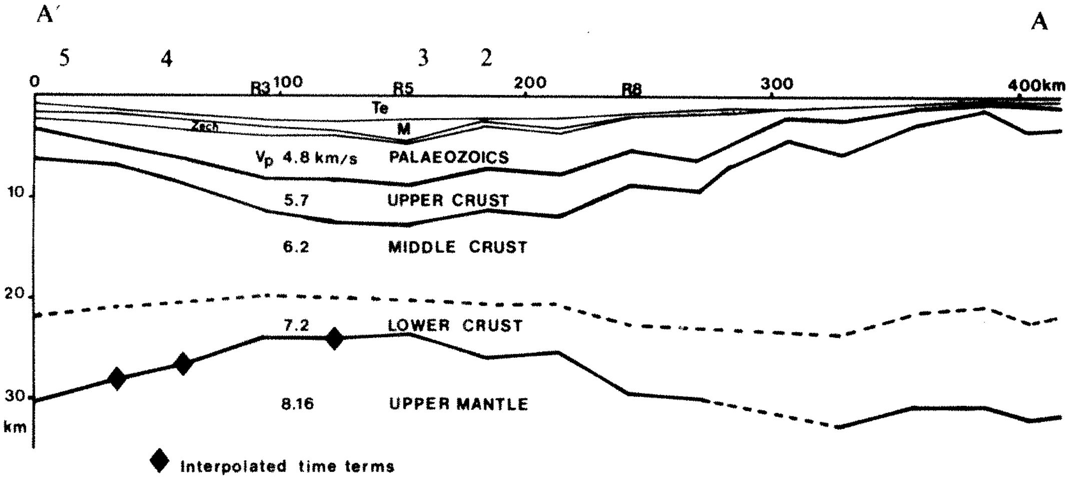

Fig. 2 Velocity depth section obtained along the main refraction profile after inverting time terms to depths. The thickness of the lower crust is constrained to be half the thickness of the middle crust as observed at the southern end of the profile[14]. The projected positions of the drill holes are shown (vertical exaggerated four times).

the model[8,16]. For a stretching factor of 2, ~1.2 km of water-filled or 3 km of sediment-filled subsidence would result. This is very close to the amount of subsidence observed.

Because of the agreement between the observed values of Moho depth, subsidence history and heat flow with the values predicted by the extensional mechanism, we would propose the following model to explain the major features of the development of the North Sea basin. The graben system was emplaced during the Permo–Triassic but with relatively little extension. From the time of the Jurassic through to the Lower Cretaceous, there was significant radial extension with the three parts of the graben system extending by a factor of ~2. The upper crust at least underwent brittle failure, thinning by a factor of ~2 and producing isostatic subsidence within the graben. When extension terminated, a general subsidence, caused by thermal relaxation through the crust, continued and the present saucer-shaped basin developed (Fig. 4). It is not clear from the observations whether the lithosphere extended as much at depth as it did at the surface. If this were the case, the ductile part of the lithosphere could have thinned by less than the brittle crust but over a greater areal extent. Such deformation would replace cold lithosphere by less dense, hot asthenosphere and would cause the graben flanking blocks to rise during extension. The consequent erosion could explain the absence of Jurassic and

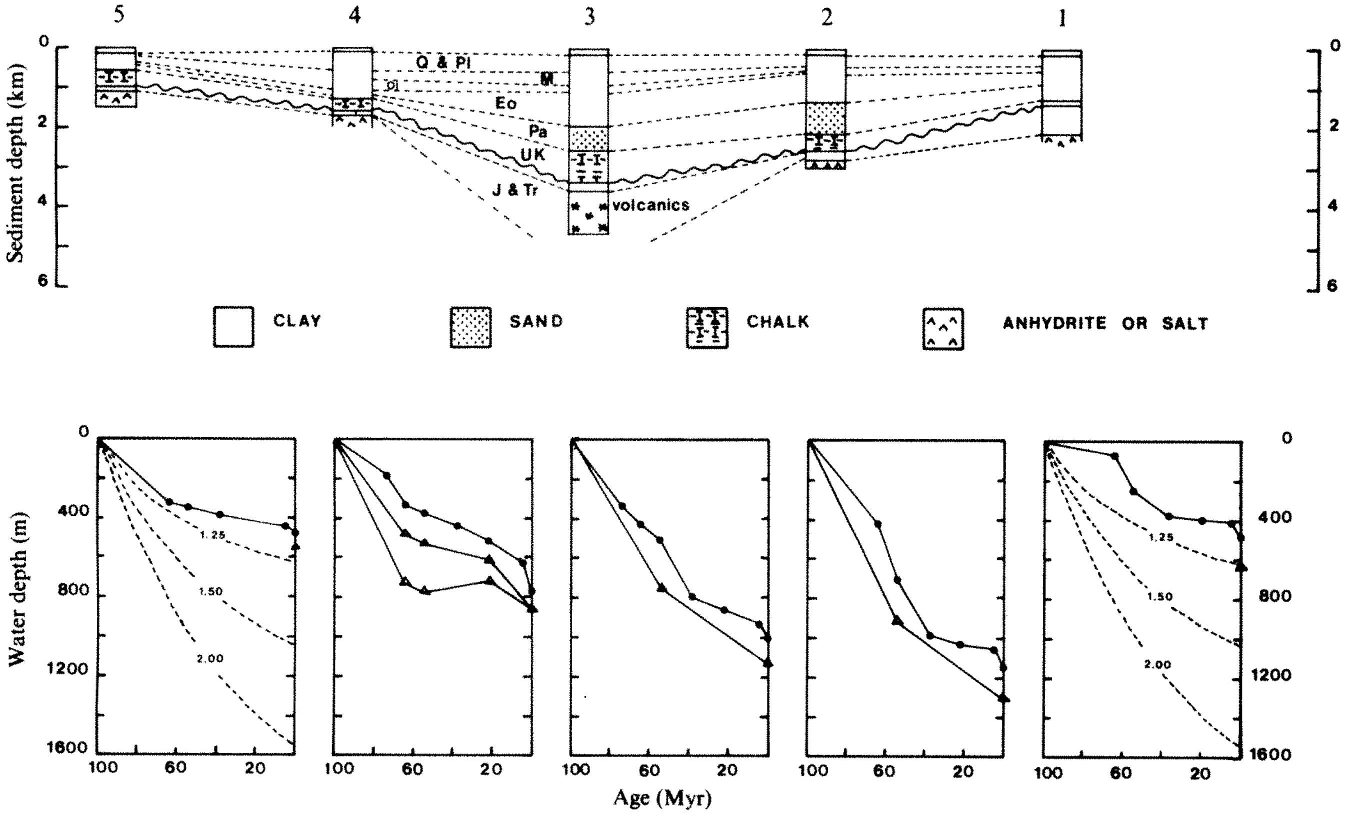

Fig. 3 A lithologic cross-section of the Witchground–Buchan Graben complex showing five wells below line AA′ on Fig. 1. The letters on the cross-section represent standard stratigraphic stages and the symbols represent the different lithologies. (Explicitly: J, Jurassic; Tr, Triassic; UC, Upper Cretaceous; Pa, Palaeocene; Eo, Eocene; Ol, Oligocene; M, Miocene; Pl, Pleistocene and Q, Quaternary.) The inferred post-Upper Cretaceous basement subsidence (●), corrected where possible for depth of deposition (△), is shown below each well. The thermal subsidence curves expected after 25% ($b = 1.25$), 50% ($b = 1.50$) and 100% ($b = 2.00$) instantaneous stretching at 100 Myr are shown on the outer two plots of basement subsidence.

Lower Cretaceous sediments on these blocks. However, the presently available seismic, eustatic and subsidence data are too imprecise to allow the inference that such sub-crustal flow has actually taken place.

This model has been proposed to account for the development of the North Sea basin in the absence of a full discussion of alternative mechanisms. We are drawn to this mechanism in preference to models invoking thermally or gravitationally induced metamorphism in the crust or upper mantle because of the observed decrease in the rate of subsidence with time. Purely flexural subsidence in response to gravitational loading predicts a deeper Moho beneath the thickest sediments, which is inconsistent with the seismic evidence. Crustal attenuation by supra-crustal erosion is rejected because of the presence of thick sequences of pre-Upper Cretaceous sediments over much of the present North Sea basin. The effects of some or all of these mechanisms may be observed in the geological record of the North Sea but we believe that the dominant mechanism in the formation of the basin is that of lithospheric attenuation due to extension.

A major responsibility of the model is to account for the required extension in the geology of the North Sea. This cannot be done by back-tracking such high angle faults as are inferred from most multi-channel seismic lines. However, hitherto unexpected low angle faults have been observed, and secondary high angle faulting is known to have occurred in the area of the Piper oilfield[19], which may be the last of a series of listric faults such as are proposed by Profett[20] to explain the extension in the Basin and Range province of the US. While this difficulty needs to be resolved, we feel that the good agreement between the refraction, heat flow and subsidence data warrants the consideration and further investigation of the model.

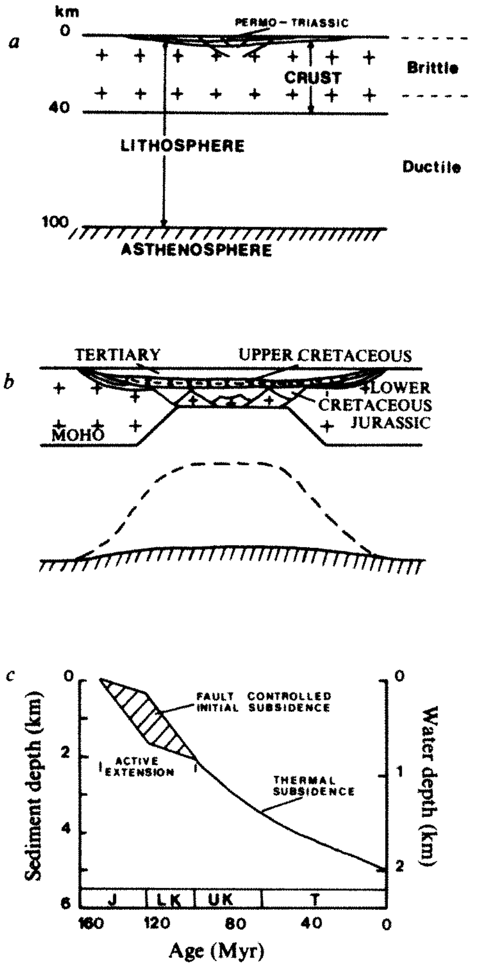

Fig. 4 An outline of the post-Triassic development of the Witchground–Buchan Graben complex. *a*, Initial cross-section before Mid-Jurassic and Lower Cretaceous extension. *b*, Present section after stretching by 100% in the graben. Note the shallow Moho, the localised Jurassic and early Cretaceous section and the widespread Upper Cretaceous and Tertiary. At the beginning of the Upper Cretaceous, the asthenosphere (dashed line) had thinned by a factor of 2. *c*, Conceptual post-Mid-Jurassic subsidence of the centre of the graben complex were the sedimentary supply to match exactly the basement subsidence.

Table 1 Comparison of observations from the centre of the graben with values predicted by the stretching model, for two stretching factors

		Model prediction	
	Observed	1.5	2.0
Fault controlled subsidence, Jurassic to Lower Cretaceous	200–800 m	800 m	1,200 m
Corrected thermal subsidence, Upper Cretaceous to Present	1,100–1,300 m	1,000 m	1,500 m
Type of thermal subsidence	Exponential	Exponential	
Moho depth below Upper Cretaceous	20 km	23 km*	17.5 km*
Heat flow (μcal cm^{-2} s^{-1})	1.70 ± 0.35	1.47 ± 0.3†	1.41 ± 0.3†

* Assuming 35 km thickness of normal crust.
† Assuming 0.8 μcal cm^{-2} s^{-1} radiogenic contribution.

Conclusions

The approach we have taken has been simplistic, and we have ignored such complications as flexure of the lithosphere due to sediment loading and eustatic sea level changes. Even though these factors have been neglected, we believe that the method is justified by the results which show that stretching by a factor of between 1.5 and 2.0 in the Jurassic and Lower Cretaceous can account for the shallow Moho, subsidence history and present heat flow across the Witchground–Buchan Graben complex. We feel that this study demonstrates the power of comparing seismic refraction and subsidence observations in unravelling the geologic history of the North Sea basin and we suggest that such comparisons may be as useful in investigating other basins. In particular, these studies may be of value to the oil industry because, if the stretching factor can be determined, it should be possible to predict the palaeo-heat flow and hence the degree of thermal maturation of the individual sedimentary horizons[16].

We thank representatives from the Shell, Conoco, Mobil and Amoco oil companies, for access to well logs which, with their experience and that of P. Ziegler, have led us through the complexities of the Mesozoic and Cenozoic sedimentary history of the North Sea.

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An immunoglobulin heavy-chain gene is formed by at least two recombinational events

Mark M. Davis*, Kathryn Calame*, Philip W. Early*, Donna L. Livant*, Rolf Joho†, Irving L. Weissman† & Leroy Hood*

* Division of Biology, California Institute of Technology, Pasadena, California 91125
† Laboratory of Experimental Oncology, Department of Pathology, Stanford Medical School, Stanford, California 94305

The events of B-cell differentiation can be reconstructed in part through an analysis of the organisation of heavy-chain gene segments in differentiated B cells. A mouse immunoglobulin α heavy-chain gene is composed of at least three noncontiguous germ-line DNA segments—a V_H gene segment, a J_H gene segment associated with the C_μ gene segment, and the C_α gene segment. These gene segments are joined together by two distinct types of DNA rearrangements—a V–J joining and a C_H switch.

THE antibody genes provide a unique opportunity for studying the molecular basis of one pathway of eukaryotic differentiation because the rearrangement of gene segments is correlated with the expression of antibody molecules. Antibody molecules are encoded by three unlinked gene families—λ, κ and heavy chain (H)[1]. The λ and κ families encode light (L) chains and the heavy-chain family encodes heavy chains. The light chains are encoded by three gene segments, V_L (variable), J_L (joining) and C_L (constant), which are separated in the genomes of cells undifferentiated with regard to antibody gene expression[2,3]. During differentiation of the antibody-producing or B cell, the V_L and J_L gene segments are rearranged and joined together while the intervening DNA between the J_L and C_L gene segments remains unmodified[2-4]. This process of DNA rearrangement is termed V–J joining. During the expression of the rearranged gene in the differentiated B cell, the coding regions as well as the intervening DNA between the J_L and C_L gene segments are transcribed as part of a high molecular weight nuclear transcript. The intervening region is then removed by RNA splicing to produce a light-chain mRNA with contiguous V_L, J_L and C_L coding segments[5,6]. Recently, we demonstrated that the heavy chain contains three analogous gene segments, V_H, J_H and C_H, which undergo a similar type of V–J joining during B-cell differentiation[7-9]. Each antibody-producing cell synthesises only one V_LJ_L polypeptide sequence and one V_HJ_H sequence, which together form the antigen-binding (V) domain of the antibody molecule.

The heavy-chain genes seem to have a special role in the differentiation of the antibody-producing cell as reflected in a second phenomenon known as the switching of heavy-chain constant regions or C_H switching. Antibody molecules can be divided into five different immunoglobulin classes—IgM, IgD, IgG, IgA and IgE—which are determined by one of eight distinct heavy chain genes [that is, C_μ, C_δ, ($C_{\gamma1}$, $C_{\gamma2a}$, $C_{\gamma2b}$, $C_{\gamma3}$), C_α and C_ϵ]. Each class of immunoglobulin seems to be associated with unique functions such as complement fixation or the release of histamine from mast cells. The process of B-cell differentiation is complex and a variety of conflicting views exists on the transitional stages. It is generally agreed, however, that IgM is the earliest immunoglobulin class that is expressed in the differentiation of a B cell (see ref. 10). The immature B lymphocyte apparently has the capacity to differentiate along a variety of discrete pathways and produce progeny which may switch from IgM expression to the expression of any one of the other classes of immunoglobulins. The terminal stage of B-cell differentiation is the plasma cell, which is committed to synthesise and secrete large quantities of a single molecular species of antibody. Several studies suggest that the specificity or V domain does not change as different immunoglobulin classes are expressed throughout this differentiation process[11-15]. During C_H switching, light-chain expression remains unaltered.

We are interested in studying the molecular mechanisms which permit a B cell (or its progeny) to express successive classes of antibody molecules. Here we demonstrate that an α heavy-chain gene derived from a terminally differentiated plasma cell is composed of three noncontiguous germ-line DNA segments. These gene segments are joined together by at least two distinct DNA rearrangements—a V–J joining and a C_H switch.

Organisation of the α heavy-chain gene is altered in differentiated as opposed to undifferentiated DNAs

We have constructed 'libraries' of recombinant phage[16] containing large (12–20 kilobases) inserts of mouse DNA in the vector Charon 4A (ref. 17). These libraries contain sufficient numbers of recombinants ($\sim 10^6$) for there to be a high probability that most single-copy sequences of a given genome are included. We have previously reported the construction of such a library from the DNA of the mouse IgA-producing myeloma tumour M603, and the subsequent isolation and characterisation of clones containing rearranged or differentiated genomic DNA. These clones, CH603α6 (α6) and CH603α125 (α125), have V_H and C_α gene segments on a single fragment of DNA[7,8]. The *Eco*RI restriction maps of these clones are shown in Fig. 1*a*. The α6 clone has three *Eco*RI fragments—one 7.3 kilobases long containing the V_H gene segment, a second of 5.1 kilobases containing the 5′ portion of the C_α gene segment, and a third of 4.4 kilobases containing the 3′ portion of the C_α gene segment. Approximately 6.8 kilobases of intervening DNA separate the V_H and C_α gene segments. The α125 clone also has three *Eco*RI fragments—one of 6.5 kilobases which is located on the 5′ side of the 7.3- and 5.1-kilobase *Eco*RI fragments also described for α6.

The genomic clones were isolated using a cloned cDNA plasmid representing the entire heavy-chain mRNA of the IgA-producing tumour S107 (denoted S107 cDNA)[7]. The S107 V_H region is about 98% homologous to the M603 V_H region at the nucleotide level[9]. Analysis by the Southern blot procedure[18,19] of *Eco*RI-digested M603 DNA hybridised to the S107 cDNA probe demonstrates the presence of three *Eco*RI fragments corresponding to those described above in the α6 clone[7]. Thus, the rearranged α6 clone is not an artefact of the cloning or isolation procedures.

When the S107 cDNA probe is used to examine a Southern blot of *Eco*RI-digested sperm or embryo DNA, a somewhat different pattern is observed[8]. In particular, the 5.1-kilobase

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*Eco*RI fragment containing most of the large intervening sequence and the 5′ portion of the C_α gene segment is not present. This observation indicates that the α6 clone is the product of one or more DNA rearrangements which presumably occurred during B-cell differentiation[8].

The α gene is composed of at least three different germ-line segments of DNA

In view of the possibility that lymphocytes earlier in the M603 lineage might first have produced IgM molecules and later IgA molecules, we decided to investigate the possible contribution of germ-line C_μ sequences as well as V_H and C_α sequences to the myeloma α gene (Fig. 1*a*). We constructed several libraries of germ-line DNA (sperm) and proceeded to isolate clones containing V_H, C_μ and C_α gene segments using cloned cDNA probes (denoted S107 V_H, C_α and C_μ) for the corresponding coding regions and the screening procedure of Benton and Davis[20]. *Eco*RI restriction maps of several germ-line clones are shown in Fig. 1*b*. We chose sperm (germ-line) DNA for our undifferentiated genomic libraries to eliminate any possibility of DNA rearrangements which may occur in somatic tissues during embryogenesis. The C_α and V_H clones seem to be representative of germ-line sequence organisation because the *Eco*RI fragments containing the corresponding coding regions (in the clones) are identical in size to those found in the Southern blot analysis of undifferentiated DNA with C_α and S107 V_H cloned cDNA probes—9.5 and 7.4 kilobases, respectively (Fig. 2*a*–*d*). The V_H and C_α probes were derived from restriction fragments of the S107 cloned cDNA[7] extending from approximately the 5′-untranslated region to codon 108 (V_H) and from codons 108 to 274 (C_α). Figure 2*a* shows that a Southern blot of a germ-line

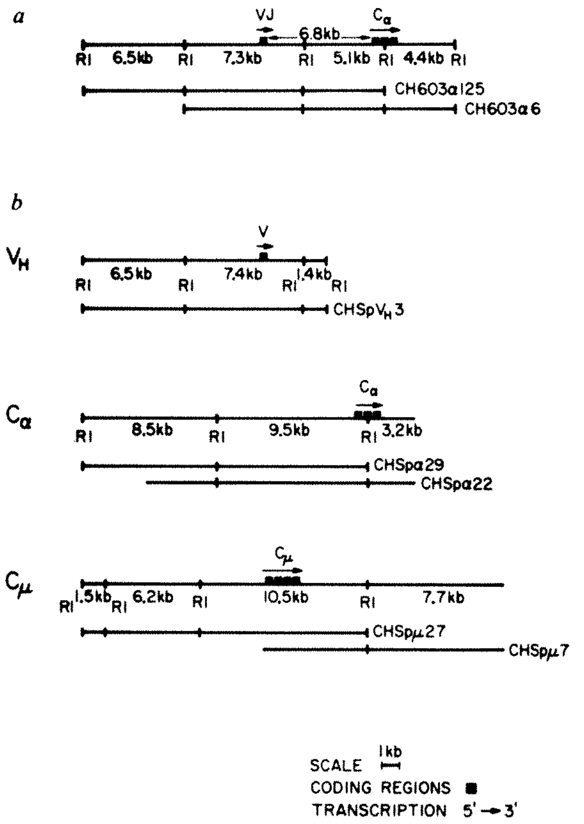
a
VJ
Cα
6.8kb
RI 6.5kb RI 7.3kb RI 5.1kb RI 4.4kb RI
CH603α125
CH603α6
b
V
VH
RI 6.5kb RI 7.4kb RI 1.4kb RI
CHSpVH3
Cα
Cα
RI 8.5kb RI 9.5kb RI 3.2kb
CHSpα29
CHSpα22
Cμ
Cμ
RI 1.5kb RI 6.2kb RI 10.5kb RI 7.7kb
CHSpμ27
CHSpμ7
1kb
SCALE
CODING REGIONS ■
TRANSCRIPTION 5′→3′

Fig. 1 *a*, α Heavy-chain clones isolated from a genomic library of myeloma M603 DNA. CH603 α6 and CH603 α125 are two overlapping clones derived from a partial *Eco*RI library of M603 DNA, constructed using the phage Charon 4A (ref. 7). The position of the respective gene segments, as well as their direction of transcription, was determined by R-loop mapping and restriction analysis[7,8]. *b*, Germ-line V_H, C_μ and C_α clones isolated from a genomic library of sperm DNA from inbred BALB/c mice. Sperm DNA[33,34] was partially digested with restriction enzymes in two ways: (1) with a mixture of *Hae*III plus *Alu*I and (2) with *Eco*RI alone. After digestion in conditions designed to maximise the yield of 12–20-kilobase fragments, these fragments were selected on sucrose gradients. The *Hae*III/*Alu*I fragments were methylated with *Eco*RI methylase and blunt end ligated with synthetic *Eco*RI cleavage sites and then cleaved with *Eco*RI essentially as described previously[16] except that *Eco*RI linker ligations were done at 18 °C to lessen the endonuclease degradation. Both types of fragments were then ligated to the isolated arms of the bacteriophage Charon 4A (ref. 16) at 4 °C. The enzymatically recombined DNAs were packaged *in vitro* using the strains of Sternberg[35] and the protocol of Hohn[36]. The efficiency of packaging was 400,000 plaque forming units (PFU) per μg inserted DNA in the case of *Eco*RI partially digested DNA and 200,000 PFU per μg for *Hae*III/*Alu*I digestions. The background of non-recombinant Charon 4A was 25% and <5%, respectively. Approximately 500,000 *Eco*RI and 1,200,000 *Hae*III/*Alu*I clones were constructed and amplified. A library of ~500,000 clones provides a 90% chance of finding a given single copy sequence and a library of 1,200,000 clones a 99% chance[37]. The use of enzymes that recognise three different sequences reduces the possibility that a particular region of interest is lost from the library because it had too many or too few restriction sites to fall within the sucrose gradient size cut. Libraries were screened[7,16] with the cDNA clone (see text) S107 or M104E C_μ cloned cDNA probes identified by DNA sequence analysis[9,38]. The C_μ clone used extends from codon 300 to the 3′-untranslated region (~1,000 base pairs).[38]. Independent overlapping clones were obtained from the C_α and C_μ gene segments. Location of the coding regions and the direction of transcription were determined by DNA sequence analyses for the CHSp PC-3 clone, by heteroduplex analyses with the α6 clone for the CHSp α29 clone, and by R-loop mapping and restriction analyses of the CHSpμ27 clone[38]. The germ-line genomic clones will be denoted μ27 (CHSpμ27), V_H3 ($CHSpV_H3$), and α29 (CHSpα29).

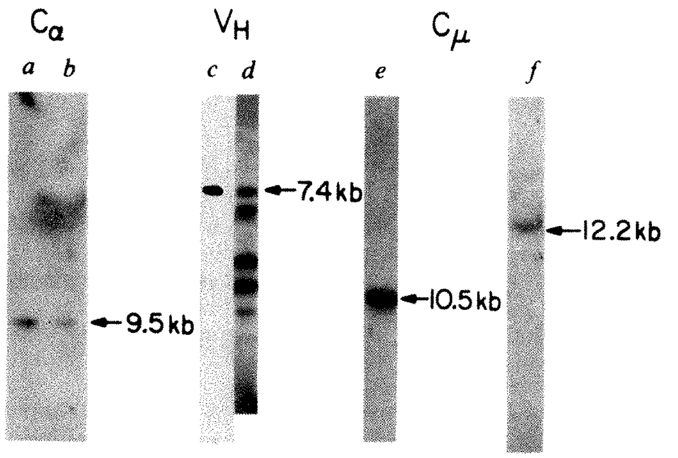

Fig. 2 Southern blot analyses of C_α, C_μ and S107 V_H coding regions in germ-line DNA. Approximately 3–20 μg of sperm DNA or 13-day embryo was digested with *Eco*RI and electrophoresed on a 0.7% neutral agarose gel for 10–12 h at 30–40 V. Gels were then blotted according to the procedures of Southern and Flavell[18,19] and hybridised with ^{32}P nick-translated cDNA[39]. Washing was for 1.5 h in 1 M NaCl, 1 M Tris *p*H 8, 0.1% SDS and 0.1% sodium pyrophosphate at 65 °C and for 1 h in 1×SSC, 0.1% SDS and 0.1% sodium pyrophosphate. Lanes *a* and *b* are α29 and embryo DNAs, respectively, hybridised to the C_α cDNA probe. Lane *c* is V_H3 DNA hybridised to the S107 cDNA probe. Lane *d* is sperm DNA hybridised to the S107 V_H cDNA probe. This probe cross-reacts with eight or nine closely related V_H gene segments[8]. Lanes *e* and *f* are μ27 and sperm DNAs, respectively, hybridised to the C_μ cDNA probe. Identical results in all cases were obtained with BALB/c 13-day embryo DNA or sperm DNA as has been found for a V_κ gene[34]. Sizes (given in kilobases) were determined by comparison with restriction fragments of PBR322 (ref. 40) or by the use of PBR322 multimers generated by *Bam*HI cleavage of PBR322 and limited ligation of the resulting monomer.

C_α clone (α29) digested with *Eco*RI and hybridised with the C_α cDNA probe yields a 9.5-kilobase fragment. A similar analysis of embryo DNA produces a band of identical size (Fig. 2*b*). Southern blots of a germ-line V_H clone (V_H3) and embryo DNA digested with *Eco*RI and hybridised to a S107 V_H cDNA probe gave, respectively, a 7.4-kilobase fragment (Fig. 2*c*) and several fragments including a 7.4-kilobase fragment (Fig. 2*d*). An important question is the relationship between the germ-line V_H3 and myeloma M603 V_H coding regions because there are at least eight V_H gene segments that hybridise to the S107 probe[8]. The germ-line V_H3 DNA sequence codes for the S107 V_H protein sequence[9] and, accordingly, differs from the M603 V_H region by a minimum of four base changes leading to three amino acid subsitutions[21]. Thus, the germ-line V_H3 and the myeloma M603 V_H gene segments may be encoded by two distinct germ-line V_H gene segments or the V_H3 gene segment may give rise to the M603 V_H gene segment by somatic mutation and selection. As we shall show subsequently, the V_H3 clone seems to be indistinguishable from the M603 clone in the V_H coding region and in more than 11-kilobases of 5'-flanking sequence by heteroduplex and restriction enzyme analyses. Therefore, our analyses of the localisation of V_H sequences in the myeloma α6 clone are valid because the germ-line V_H clone serves as a probe for the M603 V_H gene and its attendant 5'-flanking sequence.

The *Eco*RI fragment of the germ-line C_μ clone containing the C_μ coding region is smaller (10.5 kilobases; see Fig. 2*e*) than its counterpart seen on a Southern blot analysis of sperm DNA with a cloned μ cDNA probe (12.2 kilobases; see Fig. 2*f*). We believe this discrepancy arises from one (or more) deletion(s) in the DNA flanking the C_μ coding region during the propagation of the recombinant phage. In isolating μ clones from the M603 library, we obtained several 9.5–10-kilobase *Eco*RI fragments containing C_μ coding regions, whereas Southern blot analysis of M603 DNA with a cloned C_μ cDNA probe demonstrated a genomic fragment of 12.2 kilobases, as in the sperm DNA (data not shown). Attempts to isolate C_μ clones from a library of mouse liver DNA have led to similar results (N. Newell and F. Blattner, personal communication). We will present restriction enzyme data below which demonstrate that this apparent deletion in the μ clone does not affect our general conclusions.

The germ-line V_H, C_α and C_μ clones were compared with the myeloma α6 and α125 clones by heteroduplex analysis. Representative heteroduplexes from each of these comparisons show extensive homologies. The germ-line V_H clone shares approximately 11.6 kilobases of homology with the myeloma α125 clone (Fig. 3*a*). This homology extends from the 5' end of the α125 clone up to and including the V_H coding region. The germ-line C_μ clone shows 5.0 kilobases of homology with the large intervening sequence of the myeloma α6 clone (Fig. 3*b*). Starting at its 3' end, the germ-line C_α clone has about 6.4 kilobases of homology with the myeloma α6 clone (Fig. 3*c*). The heteroduplex measurements for these analyses are given in Table 1.

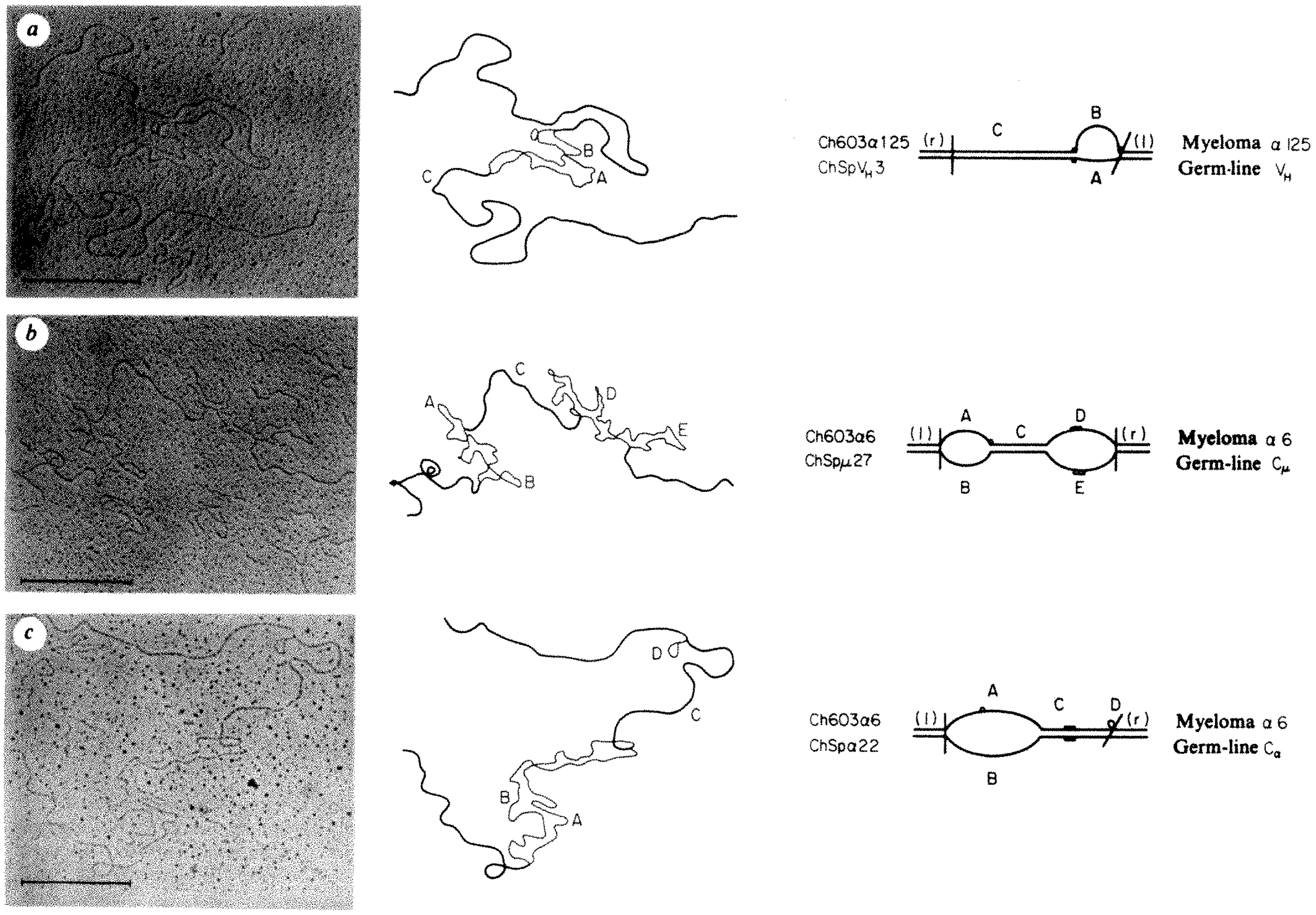

Fig. 3 Heteroduplex analyses of germ-line and somatic clones. The electron micrographs are shown on the left, tracings of these heteroduplexes in the middle and diagrammatic representations on the far right. *a*, Myeloma α125/germ-line V_H PC3. *b*, Myeloma α6/germ-line μ27. *c*, Myeloma α6/germ-line α22. Letters A–E indicate single-strand and double-strand regions for which measurements are given in Table 1. In corresponding line drawings, (r) and (l) refer to the right and left arms of the λ vector. Blocks indicate coding regions in these figures, CsCl purified phage particles were treated with 0.1 M NaOH for 10 min at 20 °C to lyse the phage and denature the DNA simultaneously. After neutralisation of the mixture, the DNA was allowed to reanneal in 50% (v/v) three times recrystallised formamide for 45 min at 20 °C before spreading for electron microscopy[41].

Table 1 Measurements of heteroduplex molecules

Heteroduplex	No. of Molecules	Distance in kilobases A	B	C^+	D	E
a						
Myeloma α125/ germ-line V_H3	35	4.5 ± 0.4	7.2 ± 0.7	11.6 ± 0.4		
b						
Myeloma α6/ germ-line μ27	30	4.8 ± 0.5	5.1 ± 0.5	5.0 ± 0.4	6.7 ± 0.4	7.5 ± 0.5
c						
Myeloma α6/ germ-line α22	26	9.3 ± 0.5	10.4 ± 0.9	6.4 ± 0.5	1.2 ± 0.2	

Measurements were standardised relative to two circular DNA molecules on the same grid [single-strand ΦX174 DNA (5,375 bases) and double-strand pBR322 DNA (4,365 base pairs)]. Letters refer to regions indicated in Fig. 3. The complete clone designations are given in Fig. 3. In heteroduplex *a*, C refers to the region of duplex between the germ-line V_H clone and the myeloma clone. A and B are the non-homologous single strands of these clones. Similarly, C for heteroduplexes *b* and *c* refers to the duplexes formed between myeloma and germ-line C_μ or C_α clones.

Comparative restriction analyses confirm and extend the heteroduplex data discussed above. A detailed restriction map for the M603 myeloma clones was obtained by double digestion with pairs of restriction enzymes and is shown in Fig. 4*a*. To compare the placement of these cleavage sites with those of the germ-line clones, the 5.1-kilobase restriction fragment of the α6 clone (Fig. 1*a*) that spans the region joining the germ-line C_α and C_μ sequences was subcloned. Using this fragment as a probe, detailed restriction comparisons of the myeloma α6 clone and the germ-line C_μ and C_α clones were made. Representative data are shown in Fig. 5 for these comparisons. Figure 5*a*, *b* and *c* represents *Hin*cII plus *Eco*RI digestions of the myeloma α6 5.1-kilobase RI fragment, the germ-line α29 clone and the germ-line C_μ clone, respectively. These digests were electrophoresed on an agarose gel, blotted onto a nitrocellulose filter and hybridised with the labelled 5.1-kilobase RI fragment. This enables homologous restriction fragments to be identified rapidly and precisely (arrows indicate identical fragments). Figure 5*d*, *e* and *f* shows a similar analysis using *Hin*dIII plus *Eco*RI digestions, respectively, of the myeloma 5.1-kilobase subclone, the germ-line α29 clone and the germ-line μ27 clone. These data, as well as additional restriction analyses of the germ-line V_H3 clone, demonstrate that 4 out of 4 restriction sites in the germ-line V_H3 clone, 10 out of 10 sites in germ-line α29 clone and 9 out of 10 sites in the germ-line μ27 clone corresponded exactly to those found in the myeloma α6 clone (Fig. 4). Not only do these restriction analyses independently confirm the heteroduplex results, but they also suggest that the component germ-line sequences of the α6 clone are very similar to their germ-line counterparts. Thus, the heteroduplex and restriction analyses demonstrate that the germ-line V_H gene segment and its 5′-flanking sequence, although not identical to its M603 counterpart, are very similar and may be used to analyse V_H gene segment organisation in the myeloma M603 clones.

DNA sequence analyses of the myeloma α6 clone and the germ-line μ27 clone have demonstrated that the heavy-chain gene family does have distinct V_H and J_H gene segments in the germ line[9]. Moreover, the germ-line J_H gene segment corresponding to that expressed in the myeloma α6 clone is associated with the germ-line C_μ gene[9]. This J_H gene segment contains the *Hha*I site that marks the end of the homology between the germ-line μ27 clone and the myeloma clones (Fig. 4). Accordingly, the distinct germ-line V_H and germ-line J_H gene segments are rearranged in the myeloma clones in a manner analogous to the V_L and J_L gene segments of myeloma light chain genes[2,3].

Although it seems unlikely, the M603 J_H gene segment and flanking sequences could have been fused to a germ-line C_μ clone as the result of some cloning artefact. To eliminate this possibility, we decided to demonstrate the germ-line association of the J_H flanking sequence and C_μ sequences by Southern blot analyses. On different slots of the same agarose gel, we electrophoresed mouse sperm DNA cleaved with either *Hin*cII or *Eco*RI, transferred these DNAs to a nitrocellulose filter and hybridised one lane of each digest with a C_μ probe and a second lane with the 5.1-kilobase *Eco*RI fragment from the intervening sequence of the myeloma α6 clone (Fig. 6). Figure 6*a* and *b* shows Southern blots of *Eco*RI-digested sperm DNA hybridised with the C_μ cDNA probe and the 5.1-kilobase *Eco*RI subclone from the α6 clone, respectively. In both lanes, a 12.2-kilobase band corresponding to the germ-line C_μ fragment can be identified. In a second digest (*Hin*cII) of mouse sperm DNA, a similar result is obtained with both the C_μ probe (Fig. 6*c*) and the 5.1-kilobase *Eco*RI subclone (Fig. 6*d*) hybridising to a 9.3-kilobase *Hin*cII fragment. The 9.5-kilobase *Eco*RI band in Fig. 6*b* and the 5.0-kilobase band in Fig. 6*d* correspond to C_α restriction fragments. In each case, one of the two bands from the 5.1-kilobase *Eco*RI probe co-migrated with the single C_μ DNA fragment. This analysis shows that at least part of the intervening sequence from the myeloma α6 clone is adjacent to the C_μ gene in the germ line. Thus, the apparent deletion in the germ-line μ27 clone is not a significant factor in our discussion.

A summary of these analyses is presented in Fig. 7. The myeloma α6 clone is composed of three noncontiguous germ-line gene segments: (1) a V_H gene segment and its 5′-flanking

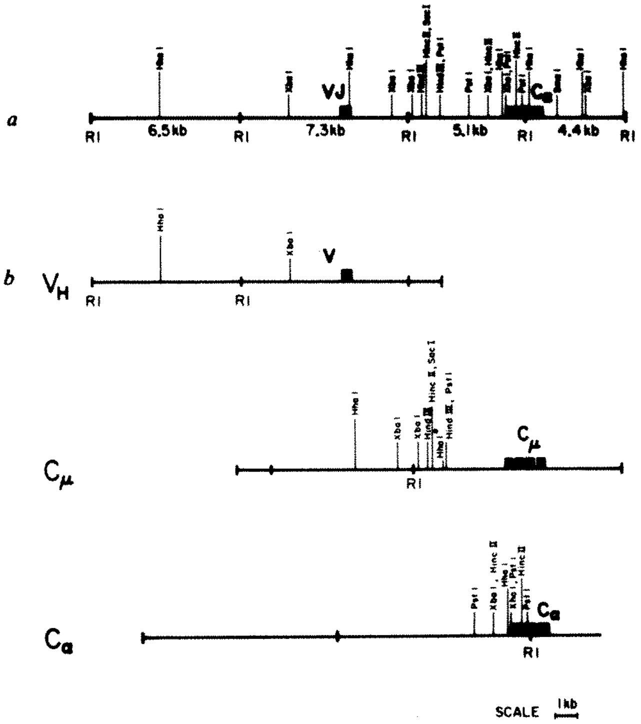

Fig. 4 *a*, Restriction map of myeloma clones α6 and α125. Specific cleavage sites were determined using double enzyme digestion and sizing by gel electrophoresis. Size standards were restriction digests of PBR322 and PBR322 multimers (see Fig. 2 legend). *Hin*dIII, *Hin*cII, *Pst*I and *Sac*I cleavage sites are only shown for the 5.1-kilobase *Eco*RI fragment. *b*, Restriction sites corresponding to those of the myeloma clones detected in the V_H, C_μ and C_α germ-line clones (Fig. 1). Restriction sites in regions corresponding to the 6.5- and 7.3-kilobase RI fragments of α6 and α125 were mapped by double digestion as above. All sites within the 5.1-kilobase RI fragment were compared by co-migration and blotting as described and illustrated in Fig. 5. The *Hha*I site marked by an asterisk in the C_μ clone was the one site found not to conform with those of the myeloma α6 clone.

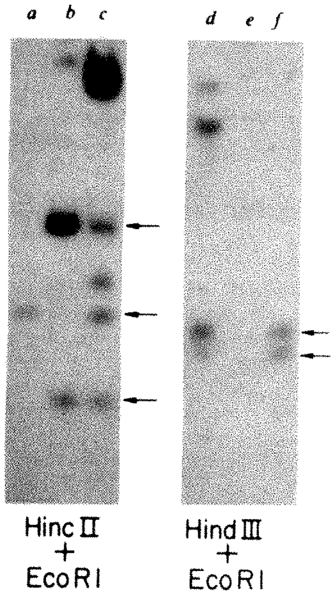

Fig. 5 Comparative restriction digests of the α6 myeloma, germ-line C_μ and C_α clones. Lanes *a–f* show parallel *Hin*cII + *Eco*RI and *Hin*dIII + *Eco*RI digests of the 5.1-kilobase *Eco*RI subclone of the α6 clone (Fig. 1*a*) and DNA from the germ-line α29 and μ27 clones (Fig. 1*b*). Samples were electrophoresed in 1% agarose, transferred to nitrocellulose and hybridised with nick-translated 5.1-kilobase α6 subclone DNA. The co-electrophoresis of restriction fragments allows a large number of different restriction sites to be compared rapidly. In this way, all 16 mapped sites in the 5.1-kilobase α6 subclone were compared with equivalent sites on the α29 and μ27 clones. Arrows show coincident bands.

sequence, (2) flanking sequences located 5′ to a germ-line C_μ gene which includes the J_H gene segment, and (3) the germ-line C_α gene segment with its flanking 3′ and 5′ sequences. Note that within the limits of the methods used here these three germ-line gene segments and their attendant flanking sequences apparently cover the entire myeloma α6 clone.

The α heavy-chain gene is formed by at least two recombinational events

Two distinct DNA rearrangements have occurred to form the M603 α gene—V–J joining and C_H switching (Fig. 8)[9]. The simplest interpretation of these observations is that the V_H gene segment is first joined to a J_H gene segment linked to the C_μ gene segment. This V–J joining, analogous to that which occurs in light chains, generates a rearranged μ gene that presumably leads to the expression of IgM molecules. V–J joining also commits an individual lymphocyte to the expression of a single V domain that remains invariant throughout subsequent steps of B-cell differentiation. Later, a C_H switch joins the V–J gene segment to the C_α gene segment to create a functional α gene and thus enables the differentiated lymphocyte to express IgA molecules. Thus, the myeloma α6 heavy-chain gene is assembled by two distinct and presumably independent DNA rearrangement events. Our heteroduplex, Southern blot and restriction mapping data show unequivocally that these two rearrangements occur at two distinct sites in the genome. These two sites of rearrangement are termed the V–J joining site and the C_H switch site, respectively.

These data are consistent with a differentiation pathway in which a B cell may switch from IgM to IgA synthesis while expressing the same V domain. We cannot establish that these two DNA rearrangement events occurred at different times, although this supposition is reasonable. We also cannot rule out the possibility of intermediate differentiation states in this B-cell lineage where IgG molecules were produced, although there is no direct evidence for such a stage.

The C_H switch may be explained by any one of several genetic models

Several mechanisms of C_H switching have been proposed[11,22–26], many of which are similar to those proposed for V–J joining[27]. These models can be categorised as involving either DNA rearrangements to replace one constant region with another (successive deletions, excision–insertions or inversions) or the differential processing of a large nuclear RNA transcript containing multiple heavy-chain constant-region genes[26].

The RNA processing model seems unlikely as a general mechanism for C_H switching at the level of antibody-secreting plasma cells. High molecular weight nuclear RNAs from three myeloma tumours hybridise only with a cDNA probe complementary to the class of immunoglobulin expressed in that tumour and not with probes from non-expressed immunoglobulin classes[28]. Moreover, cell fusion experiments hybridising two different myeloma cells demonstrate that the hybrid cells synthesise only parental V_HC_H combinations[29], a result in conflict with the simple RNA processing model. Furthermore, neither $C_{\gamma1}$ nor C_μ DNA probes hybridise on Southern blots with the M603 α clones (data not shown), contrary to one prediction of the RNA processing model.

On the other hand, the evidence presented here strongly supports DNA rearrangement as a fundamental element in the mechanism for heavy-chain switching. As summarised in Fig. 7, a very large segment of C_μ flanking sequence has been brought adjacent to C_α and V_H gene segments in the active α gene of myeloma tumour M603. The creation of the M603 α gene apparently requires two DNA rearrangements (Fig. 8). A C_H switching

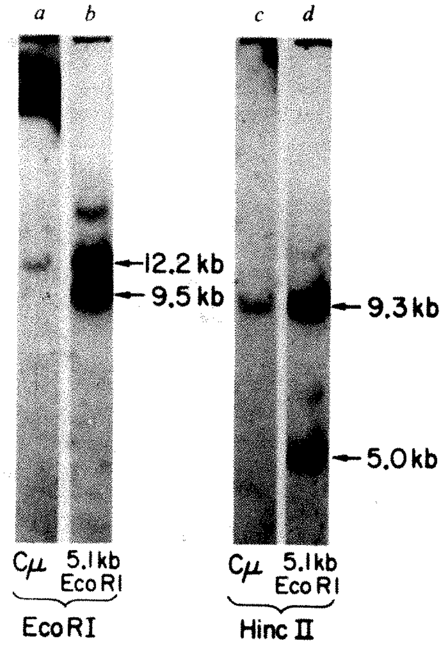

Fig. 6 Southern blots of the 5.1-kilobase *Eco*RI and C_μ fragments. Mouse sperm DNA was digested with *Eco*RI or *Hin*cII and 3 μg was loaded onto a 4 mm × 20 × 20 cm 0.7% agarose gel, electrophoresed at 40 V for 10 h and blotted as described. Probes used were either the 5.1-kilobase *Eco*RI fragment or a C_μ cDNA clone nick-translated to 4×10^8 c.p.m. per μg. Washing was as described in Fig. 3 legend except that lanes hybridised with the 5.1-kilobase *Eco*RI fragment were washed further in 10 mM NaCl, 10 mM Tris, 0.1% SDS and 0.1% $NaPP_i$ for 2 h at 68 °C to reduce the signal strength of weakly homologous repeats. Filters were exposed for 12 h with an intensifying screen at −80 °C. The faint band above the 12.2-kilobase band in *b* is a partial digestion product. All lanes shown were run on the same gel and blotted simultaneously, alignment being assisted by inclusion of pBR322 multimers as internal standards (see Fig. 2).

DNA rearrangement is not postulated in the RNA processing models[26]. The data presented here do not distinguish between the various types of DNA rearrangements proposed, but the hybridisation kinetics experiments of Honjo and Kataoka are consistent with a deletional mechanism for the C_H switch[25]. In addition, recent experiments suggest that V–J joining in mouse λ genes is accomplished by a deletional mechanism[4]. Thus, both types of DNA rearrangement, V–J joining and C_H switching, may arise through deletional mechanisms. If the deletional model is correct for either type of DNA rearrangement, the differentiation of B cells is irreversible because chromosomal information is lost with the excision of each deletional loop of chromosome.

Gene organisation studies may delineate distinct pathways of B-cell differentiation

It will be interesting to determine whether all joined α genes have the same switch site. Southern blot analyses of the DNA from a second IgA-producing myeloma tumour, H8, with the 5.1-kilobase *Eco*RI probe yield a restriction fragment pattern identical to that of M603 DNA (data not shown). In particular, the 5.1-kilobase *Eco*RI fragment (Fig. 1) which contains the switch site seems the same. These data suggest that the expressed α genes in both M603 and H8 myeloma tumours have the same C_H switch site. However, Southern blot analyses of several other closely related IgA-producing tumours do not show a 5.1-kilobase *Eco*RI fragment (M.M.D., unpublished observation). Thus, there may be multiple C_H switch sites for the C_α gene segment. As it seems that B cells producing IgM or IgG may switch to the production of IgA, perhaps distinct C_H switch points reflect distinct pathways of B-cell differentiation. It will also be interesting to determine the location and number of switch sites for other immunoglobulin classes. If each C_H gene segment has a unique site or set of sites for C_H switching, one may be able to trace the distinct pathways of B-cell differentiation by studying the sequence organisation of each functional heavy-chain gene.

DNA rearrangements of antibody gene segments lead to combinatorial amplification of immunoglobulin information

V–J joining and C_H switching are mediated by DNA rearrangements which display combinatorial properties that amplify the germ-line information encoding the antibody gene families. (1) The V and J gene segments of one antibody gene family may be joined in a combinatorial manner to generate diversity in the third hypervariable regions of both κ and heavy chains[4,8,9,30,31]. For example, mice may have at least 200 V_H and 5 J_H gene segments that may be joined combinatorially to generate 1,000 different V_HJ_H coding regions. (2) One V domain may be combinatorially switched among eight or more different C_H regions to carry out a variety of different effector functions that are directed at eliminating antigen or triggering defensive mechanisms such as complement fixation. Thus, each recognition (V) domain may be switched to many different effector (C) domains. The combinatorial properties of antibody gene segments and polypeptides therefore contribute to several fundamental aspects of the vertebrate immune response—V region diversity and the combinatorial switching of antigen-recognition (V) domains with a variety of different effector (C) domains during B-cell differentiation. Other complex eukaryotic gene families may use similar DNA combinatorial mechanisms for information amplification[32].

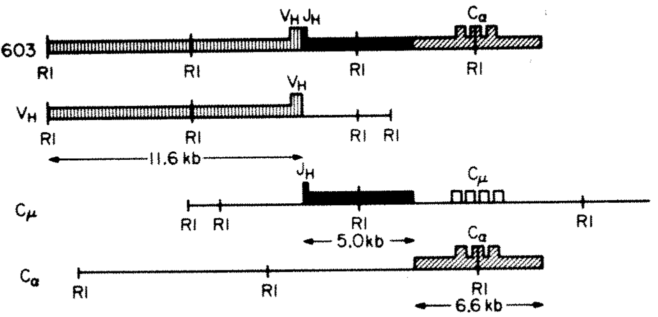

Fig. 7 Origins of the three germ-line components of the myeloma α heavy-chain gene. Various types of shading indicate homology by heteroduplex analyses and by restriction enzyme analyses.

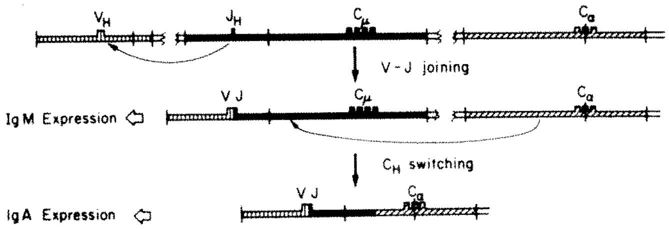

Fig. 8 Two types of DNA rearrangements leading to the creation of the myeloma α heavy-chain gene V–J joining and C_H switching. V–J joining indicates a DNA rearrangement that joins the V_H and J_H gene segments. Because the J_H segments seem to be associated with the germ-line C_μ gene[9], V–J joining permits a μ chain and IgM molecules to be expressed by the differentiating B cell. C_H switching denotes a DNA rearrangement that replaces the C_μ gene segment with a C_α gene segment. This second rearrangement presumably permits an α chain and IgA molecules to be expressed by the now fully differentiated lymphocyte.

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The primary structure of 16S rDNA from *Zea mays* chloroplast is homologous to *E. coli* 16S rRNA

Zs. Schwarz[*] & H. Kössel[†]

Institut für Biologie III der Universität Freiburg, Schänzlestrasse 1, D-78 Freiburg i. Br., FRG

The nucleotide sequence of ribosomal DNA coding for 16*S rRNA from* Zea mays *chloroplast has been determined. A comparison with the* 16*S rRNA sequence from* Escherichia coli *reveals strong homology and thereby demonstrates the prokaryotic nature of chloroplast ribosomes from a higher plant.*

CHLOROPLASTS of green plants contain 70S ribosomes which differ from the eukaryotic 80S type ribosomes of the cytoplasm[1]. The organelle-specific ribosomes mediate translation of organelle-specific mRNAs, which in turn are encoded in chloroplast DNA[2,3]. Organelle-specific rRNA[4] and tRNA[5] species are also encoded in the chloroplast genome, which thus provides the basis for the semiautonomous nature of the organelle. This, along with the various prokaryotic characteristics of the chloroplast-specific translation system, has lent strong support to the endosymbiont hypothesis[6]. This hypothesis postulates that modern chloroplasts are descendants of endosymbiotic prokaryotes—particularly cyanobacteria[7]—which have entered eukaryotic cells during evolution.

Due to a supposedly heavy functional constraint of a highly complex entity such as the ribosome, it seemed likely that its components would have changed comparatively slowly during evolution. Therefore, a comparison of rRNA primary structures from chloroplasts and *E. coli* should provide a quantitative measure of their evolutionary relationship, even if they are separated by a large phylogenetic distance. As an extension of previous work based on T_1-oligonucleotide catalogues[7-9] of 16S rRNAs, complete sequencing should not only allow the exact positioning of conserved T_1 oligonucleotides, but also a comparison of regions in which larger T_1 oligonucleotides are absent. Furthermore, a differentiation between strongly conserved regions and regions which have gone through changes during the course of evolution will be possible only on the basis of complete sequences. We have, therefore, analysed rDNA from *Zea mays* chloroplasts at the nucleotide level, which should allow deduction of the primary structures of the corresponding rRNA species and comparison with the corresponding rRNA species from *E. coli*. Furthermore, it was anticipated that analysis of sequence homology between organelle and *E. coli* rRNA species would support the prediction of secondary structures if they are common or would help to exclude them if they are not preserved. In this article we report the primary structure of 16S rDNA from *Zea mays* chloroplast and compare it with *E. coli* 16S rRNA recently analysed by others[10,11].

Sequencing of maize chloroplast 16S rDNA

DNA from *Zea mays* chloroplasts is a circular molecule of about 135 kilobase pairs on which two rRNA coding regions are positioned in opposite orientation within two 22 kilobase-pair inverted repeats[4]. Each inverted repeat includes within a 12 kilobase-pair *Eco*RI fragment one copy of a 16S rRNA, 23S rRNA, 4.5S and 5S rRNA gene and a 2 kilobase-pair spacer region between the 16S and 23S rRNA genes. One such 12 kilobase-pair fragment has been linked to the plasmid vector pMB9 within the *E. coli* clone pZmc134 (ref. 4). This clone was therefore used for isolation, mapping and sequencing of the 16S rRNA coding DNA fragments, which are positioned within the left half of fragment *Bam*HI·C (Fig. 1)[4,12,13].

In Fig. 2 the RNA-like strand of the entire 16S rRNA gene from maize chloroplast is depicted (lower row of each line). Together with maize chloroplast 4.5S and wheat 5S rDNA sequences (T. Dyer *et al.*, unpublished) it represents—to our knowledge—the first sequence analysis of a complete gene originating from a higher plant.

The exact termini of the structural part of the gene cannot be located by DNA sequencing and must await sequencing of the RNA ends. Taking into account the inhomology of the first four positions, it seems not unlikely that the position of the 5′-terminal nucleotide of maize 16S rRNA is different from position 1, although very close to it. At the opposite end positions 1,540 (last homologous position) or 1,541 (non-homologous, but in accordance with the chain end of *E. coli* 16S rRNA) appear as the most likely candidates coding for the 3′-terminal nucleotide of the chloroplast 16S rRNA. Neglecting this uncertainty a chain length of 1,491 results, which, due to several deletions, is 50 residues shorter than the *E. coli* 16S rRNA.

Sequence homology between maize chloroplast and *E. coli* 16S rRNA

When the maize chloroplast 16S rDNA sequence is compared with the 16S rDNA[10] or rRNA[11] from *E. coli* (Fig. 2) extensive

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[*] Present address: Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138.

[†] To whom correspondence should be addressed.

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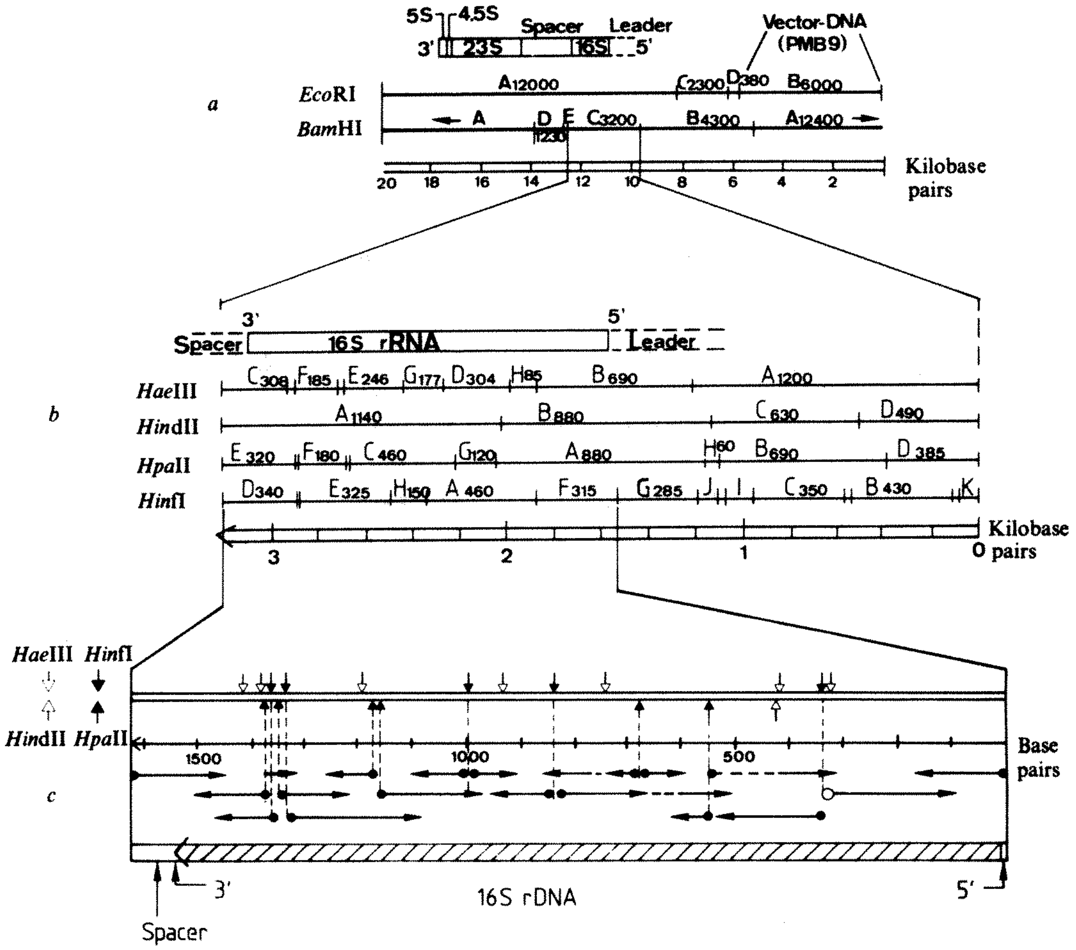

Fig. 1 Physical mapping and use of restriction fragments for sequence analysis of 16S rDNA from *Zea mays* chloroplasts. In *a* the *Eco*RI and *Bam*HI cleavage sites are shown relative to the position of cloned rDNA from maize chloroplasts (fragment *Eco*RI·A) and to the pMB9 vector DNA (fragment *Eco*RI·B)[4,12]. Fragment *Bam*HI·E originally placed between fragment C and B[12,13] has now been positioned between fragments D and C (W. Koch and H. K., unpublished). *b* Represents the fine mapping of fragment *Bam*HI·C, which contains the entire 16S rDNA within its left half. It should be noted that our previous map[12] has been slightly revised in respect to the position of fragments *Hin*fI·F/G and that fragments *Hae*III·C/D have now been assigned by sequence overlaps. In *c* the individual fragment ends selected for terminal labelling and sequence analysis of the respective fragments are indicated by filled circles (5'-terminal labelling) or open circles (3'-terminal labelling). Sequencing was carried out according to the dimethylsulphate/hydrazine procedure[27] with minor modifications as described[28]. The solid part of each connecting arrow represents portions of terminally labelled single fragments from which unambiguous sequences could be obtained. The sequences of most of the fragments have been worked out by a series of overlapping gels (not individually indicated) which allows multiple reading of more than 90% of all positions. The portions of antiparallel overlaps from which sequences for both strands can be deduced can also be seen. Single terminally labelled fragments were obtained from fragments labelled on both ends either by secondary cleavage reactions where sites were available or by strand separation.

homology is observed. Out of the 1,541 residues from *E. coli* 16S rDNA 1,144 positions (74%) are identical in maize 16S rDNA. The maximum number of consecutive identical nucleotides is 53 (positions 916 to 968). Note that in spite of the high degree of homology only less than one third of the cleavage sites for restriction enzymes are identical. Contrary to the structural part of the cistron, an immediately adjacent spacer region comprising nucleotide positions 1,542 to 1,729 shows virtually no homology with its *E. coli* counterpart[13]. Similar divergence has been observed with other spacer sequences[14]. This is evidence for a heavy constraint having acted in favour of the conservation of the ribosomal components during evolution, while an apparently free drift has been possible for the spacer region.

Of the 10 or 11 methylated positions observed in *E. coli* 16S rRNA[10,11] only position 1,206 is not preserved in the maize sequence. All others are identical and embedded within highly homologous regions (Fig. 2). From this a nearly identical methylation pattern can be predicted for chloroplast 16S rRNA if methylation enzymes of equal specificity are preserved in the organelle.

Sequence homology of the two 3'-terminal regions suggests[13] that initiator regions of chloroplast mRNAs will show typical prokaryotic sequences such as GGAGGA and its variants[15,16]. This is supported by the observation that an *in vitro* system from *E. coli* can be used for efficient translation of higher plant chloroplast mRNAs[17,18]. Sequences near the 3' end of *E. coli* 16S rRNA (positions 1,501 to 1,509 and 1,524 to 1,533) have been postulated as being involved in binding of the 50S ribosomal subunit by means of their complementarity to the 3' end of 23S rRNA[19]. This postulate gains support from the observation that these sequences have been largely preserved (except for the two positions 1,507 and 1,526) in maize 16S rRNA. It remains to be seen, however, whether the 3' end of maize 23S rRNA has also preserved complementary sequences and whether complementary substitutions have taken place in the positions corresponding to positions 1,507 and 1,526 of 16S rRNA.

Deletions reduce possible hairpin structures

Due to several deletions the actual chain length of maize chloroplast 16S rRNA is about 50 residues shorter than the *E. coli* species. Apart from several single nucleotide deletions occasionally compensated for by single nucleotide insertions at nearby positions (for example, positions 624/630 or 833/839), three regions containing larger deletions (Fig. 2, positions 77–99, 233–240 and 452–477) are observed within the 5'-terminal third of the cistron. Note that these deleted regions are flanked by conserved regions (with the exception of the left border of the 233–240 deletion) and that due to two of the deletions possible hairpin structures (Fig. 3*a*, *b*) present in *E. coli* 16S rRNA are precisely cut out. Independent evidence for a possible hairpin structure comprising positions 452 to 477 of *E. coli* 16S rRNA (Fig. 3*b*) has recently been deduced from co-migration of oligonucleotides representing this region in a two-dimensional gel electrophoretic system[20]. However, in contrast to Fig. 3*a*, positions 233–240 were predicted to base-pair with positions 122–126. Taking into account that due to the 233–240 deletion one complement of this secondary interaction is completely missing from maize chloroplast 16S rRNA, we consider this possibility unlikely. More likely is a structure in which the base-paired stem of Fig. 3*a* is connected by a continuous stack to a base-paired region comprising positions 241–294 (see Fig. 6 of ref. 20). A further possible hairpin structure is only reduced in size due to two closely positioned deletions (Fig. 3*c*).

It will be interesting to see whether similar deletions are present in other chloroplast 16S rRNAs and if the resulting, apparently more primitive structures are accompanied by smaller sizes of other ribosomal components or by even smaller numbers of ribosomal proteins. (The recent observation of an intervening sequence in the *Chlamydomonas* 23S rRNA gene[21] does not necessarily contradict a more primitive nature of chloroplast rRNA genes, if intervening sequences are interpreted as evolutionary remnants.)

Possible common secondary structures contain compensating base changes

Computer-aided screening of the maize chloroplast 16S rRNA for possible hairpin structures leads to a very large number of hypothetical secondary structures which are difficult to evaluate *per se*. Considering, however, the *E. coli* 16S rRNA species as a not too distantly related mutant species (or vice versa), screening for secondary structures can be narrowed down to structures which are common to both species. Also, common secondary structures gain even higher probability if compensatory base changes are observed in distant positions of the primary structure as exemplified in Fig. 4.

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A loop structure near the 5′ terminus has been postulated for *E. coli* 16S rRNA[22]. The homologous structure of maize chloroplast 16S rRNA shown in Fig. 4*a* contains six base exchanges (as well as one deletion and one insertion, which are considered as neutral) which match each other to allow a loop structure of almost identical size and stability as in the *E. coli* species. We have recently described similar conservation of a 3′-terminal loop structure[13]. In Fig. 4*b* a double loop structure from the middle portion of 16S rRNA is proposed which also includes a far distance pairing between positions 580–587 and 878–884. This domain would contain altogether 11 exchanges of complete base pairs, that is 22 single base substitutions; although the individual exchanges have occurred at comparatively distant positions of the primary structure, they exactly compensate each other and thereby permit maintenance of a thermodynamically highly stable secondary structure of similar size. Such compensatory base exchanges, therefore, give strong support to the functional significance of the proposed secondary structures, although they would have to be confirmed by more direct experimental evidence. Additional support for the existence of the double hairpin region shown in Fig. 4*b* comes from kethoxal reaction data[23], which indicate sensitivity of the G residue at position 790, and from T_1 RNase sensitivity data[24], which show that the two loop regions are sensitive within the free 30S particle. A reduced sensitivity against kethoxal and T_1 RNase in the presence of coupled 50S particles has led to the prediction[23,24] that this domain is involved in the interaction of the 30S and 50S subunits at their interphase. In agreement to this, 30S particles modified at position 790 are not found within the 70S particles obtained from subsequent coupling[25]. From the present data it seems likely that the subunit interaction is mediated by the 768–884 domain of 16S rRNA in the form of

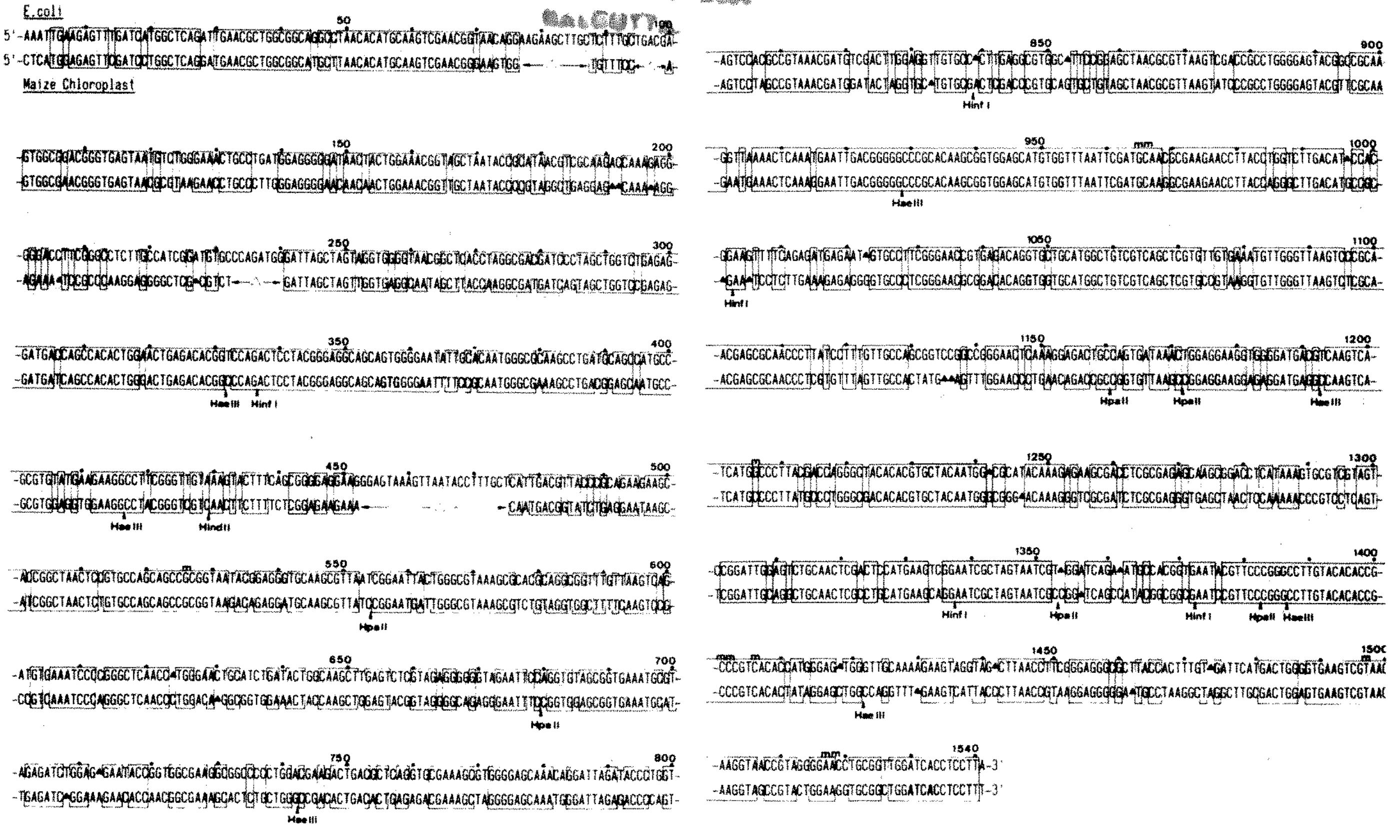

Fig. 2 Nucleotide sequence of 16S rDNA from *Zea mays* chloroplasts. Only the non-coding, RNA-like strand is given in the lower row of each line. The RNA-like strand of *E. coli* 16S rDNA[10,11] is depicted on top of it for comparison. Homologous sequences are marked by boxes. Triangles within sequences symbolise deleted positions as compared to the other DNA. Numbering refers to *E. coli* 16S rDNA. Due to several deletions not all *E. coli* positions are represented in the maize sequence. The actual numbering of the maize rDNA sequences would therefore be correspondingly lower if this sequence would be counted continuously. It should also be noted that the maize chloroplast rDNA sequence is depicted in opposite orientation as compared to Fig. 1. Cleavage sites for the restriction enzymes used in Fig. 1 are indicated. Methylated positions of *E. coli* 16S rRNA are marked by m.

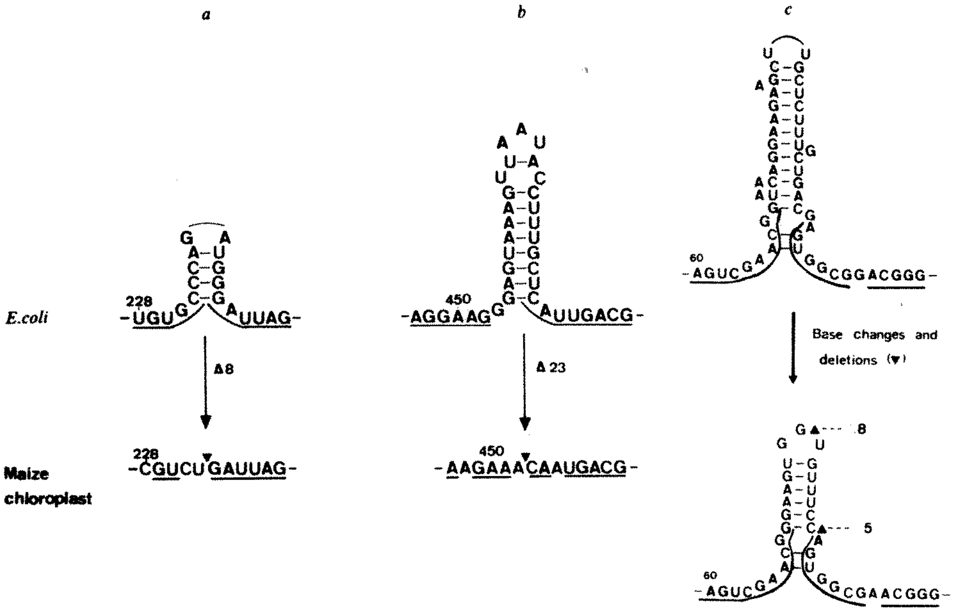

Fig. 3 Postulated hairpin structures deleted (*a*, *b*) or reduced (*c*) in maize chloroplast 16S rRNA as compared to *E. coli* 16S rRNA. Numbers refer to positions in *E. coli* 16S rRNA (see Fig. 2). Homologous positions are marked by underlining. Positions of deletions are indicated by ▼.

the hairpin structures depicted in Fig. 4*b*. Co-migration of oligonucleotides in a two-dimensional electrophoretic system also supports the existence of secondary structure in the 808–997 region of isolated *E. coli* 16S rRNA[20]. From these data, however, a secondary structure very different from Fig. 4*b* was proposed (see Fig. 6 of ref. 20). On the basis of best fit for compensatory base exchanges between *E. coli* and maize chloroplast 16S rRNA we prefer the structure proposed in Fig. 4*b*.

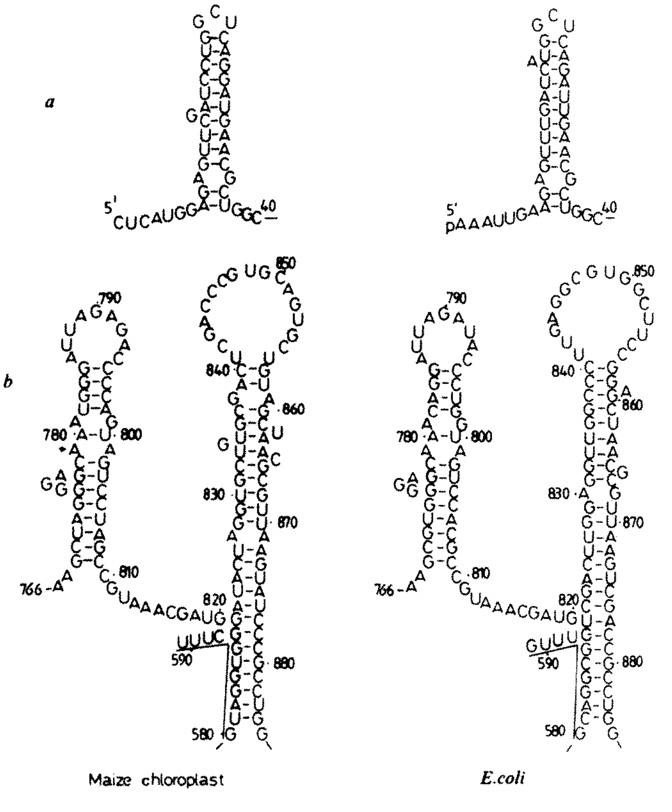

Fig. 4 Postulated secondary structures of 16S rRNA regions which, due to homology and/or compensating substitutions in distant positions of the two chains, show nearly identical dimensions. The two structures to the left represent maize chloroplast rRNA sequences. A possible far distance interaction in *b* is marked by underlining. Numbers refer to positions of *E. coli* 16S rRNA (see Fig. 2).

Compensatory base exchanges also support the proposed secondary structures for the protein S8/S15/16S rRNA interacting site[26] and allow a slight refinement of one of the two proposed structures (not shown here, but compare Fig. 7 of ref. 26 with our Fig. 2).

These examples taken together demonstrate that compensating base substitution between functionally related RNA species can provide a valuable criterion for the evaluation of their hypothetically or experimentally deduced secondary structures.

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Note added in proof: A major portion of the yeast 18S rDNA has recently been sequenced[29]. A comparison of this sequence with 16S rDNA from either *E. coli* or maize shows only very few homologous regions; this indicates a wide evolutionary distance between prokaryotic 16S and eukaryotic 18S rRNA and further substantiates the prokaryotic character of the maize 16S rDNA.

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Left-handed DNA helices

Struther Arnott, Rengaswami Chandrasekaran, D. L. Birdsall, A. G. W. Leslie*
& R. L. Ratliff†
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

The synthetic DNA polymer poly d(GC) · poly d(GC) has been studied by X-ray diffraction of orientated fibres in which it has either the well known (A or B) right-handed conformations or a new left-handed helical conformation. Similar left-handed conformations have been observed with poly d(AC) · poly d(GT) and with poly $d(As^4T)$ · *poly* $d(As^4T)$, *demonstrating that the novel structure is accessible to any DNA segment with an alternating purine–pyrimidine base sequence.*

SINGLE crystals of $d(CpG(pCpG)_2)$ complexed with spermine and of the high salt form[1] of d(CpGpCpG) contain duplex structures which, if elaborated in a polymer, would lead to a left-handed DNA double helix of pitch ~4.4 nm containing six dinucleotide pairs (A. Rich and R. E. Dickerson, personal communications). Using X-ray diffraction of oriented fibres, we have been studying the structures of sodium salts of poly d(GC) · poly d(GC), a synthetic DNA polymer of high molecular weight ($\sim 2\times 10^6$). The polynucleotide is usually found to have one of the common (A or B) right-handed double helical conformations. Occasionally it is found with what must be essentially the same left-handed double helical conformation observed in the single crystal studies: its unit of structure is a dinucleotide, its screw symmetry is 6-fold, and its pitch is 4.35 nm. Moreover, our polymer models which incorporate the same *syn*-G and *anti*-C nucleoside conformations observed by the MIT group give a good account of the fibre diffraction intensities (F. J. Kolpac, A. Rich and G. J. Quigley, personal communications).

The left-handed helices can appear in fibres of poly d(GC) · poly d(GC) in which previously the right-handed B-DNA conformation was observed, and in conditions quite unexceptional with respect to hydration or the type or number of associated cations. Similar structures are observed with polymers containing other alternating purine–pyrimidine base sequences. It would be surprising, therefore, if this novel DNA conformation were not used biologically.

Molecular dimensions and packings

The A form of poly d(GC) · poly d(GC) (Fig. 1*a*) is not detectably different from that of other DNAs (ref. 2) being an 11_1 helix of pitch 2.82 nm packing in a monoclinic (*C2*) unit cell with $a = 2.22$ nm, $b = 4.06$ nm, $c = 2.82$ nm, $\beta = 97°$.

The B form (Fig. 1*b*) has the same symmetry (10_1) and helix pitch (3.35 nm) as in other DNAs (ref. 3). The overall intensity distribution in the diffraction pattern also suggests that the normal B-DNA conformation is unchanged although the orthorhombic unit cell with $a = 3.61$ nm, $b = 3.79$ nm, $c = 3.35$ nm probably contains twice as many DNA chains as the usual cell.

The new form (Fig. 1*c*) at 44% relative humidity is a 6-fold helix which packs in a hexagonal unit cell with $a = b = 1.91$ nm, $c = 4.35$ nm. The axial translation per (dinucleotide) residue is therefore $h = 0.725$ nm.

The conformation of left-handed DNA

The structures of about two dozen right-handed polynucleotide helices have been examined in detail[4]. All of them are conformationally polymononucleotides. The novel form of poly d(GC) · poly d(GC) is a polydinucleotide conformationally as well as chemically. Using the linked-atom least-squares procedure[5,6] and assuming that the G and C nucleosides have *syn* and *anti* conformations respectively (as found by the MIT group), we have produced a preliminary model for this structure (Table 1 and Figs 2 and 3) with standard bond lengths and bond angles and a minimum of steric compression. One nucleotide (pC) has the same kind of conformations that are commonly observed in right-handed polynucleotide helices such as A- and B-DNA. The nucleotide pG is quite different not only in the mutual orientation of sugar and base rings but also in the C4′–C5′ conformation which is *trans* rather than *gauche*$^+$, and the P–O conformations which are *gauche*$^+$ rather than *trans* or *gauche*$^-$. Nevertheless, this combination of conformation angles yields a structure free of overshort nonbonded contacts.

Morphology

In a polymononucleotide duplex every nucleotide is quasi-symmetrically related to every other one by the operations of the molecular screw axis or the dyad axes in the mean plane of each base pair and midway between each pair. One consequence is that the normals to every base plane make equal angles (γ) to the helix axis. In a polydinucleotide duplex like the novel left-handed DNA this is no longer the case and the guanines ($\gamma_G = 18°$) have quite a different orientation to the cytosines ($\gamma_C = 7°$).

The angle between the guanine and cytosine planes of a base pair (14°) is similar to that found in A-DNA (10°) and B-DNA (13°). The twist axis is tilted 5° from being perpendicular to the helix axis. This tilt is in the same sense as in all members of the B genus of polynucleotide helices[4]. The position of the base pairs relatively close to the helix axis is also like that found in the B genus. Consequently both major and minor grooves are deep. The base pairs are well stacked. As is commonly the case in polynucleotide duplexes most of the overlap involves the central part of the base pairs and not overlap of the heterocyclic rings themselves (Fig. 2). The cytosines are sandwiched relatively effectively between the guanines but these are more exposed.

Another consequence of the molecular asymmetric unit being a dinucleotide is that successive phosphate groups do not have the same radius (R): in CpG $R_P = 0.95$ nm; in GpC $R_P = 0.85$ nm. Nor are the phosphates equally spaced along the polynucleotide chain: in pGp $d(P\ldots P) = 0.61$ nm, in pCp $d(P\ldots P) = 0.64$ nm. As a result of this, pairs of proximate phosphates from the antiparallel chains cluster together (Fig. 3). Effective charge neutralisation or shielding is thus important to maintain this structure.

The provenance of the left-handed form of poly d(GC) · poly d(GC)

It is well known[10,12] that an unusual structure is accessible to poly d(GC) · poly d(GC) and its fragments in aqueous solutions of higher concentration than 2.5 M in Na^+ or >0.7 M in Mg^{2+}. The predictions of Patel *et al.*[10] with regard to the details of this unusual structure are sufficiently fulfilled by our model for

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* Present address: Biophysical Section, Blackett Laboratory, Imperial College, Prince Consort Road, London SW7 2BZ, UK.

† Permanent address: University of California, Los Alamos Scientific Laboratory, PO Box 1663, Los Alamos, New Mexico 87544.

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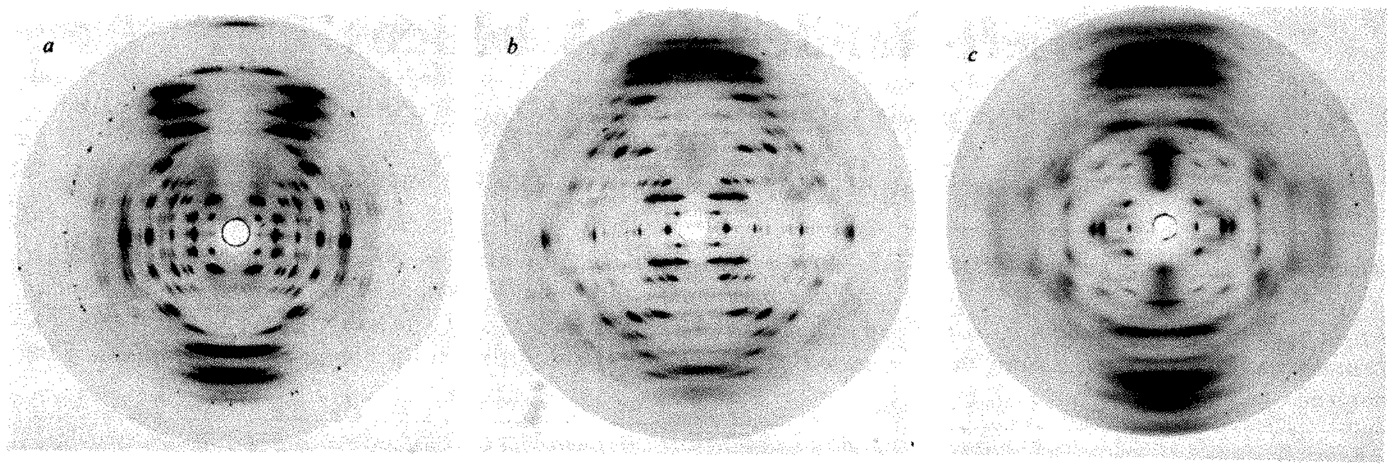

Fig. 1 X-ray diffraction patterns from oriented fibres of poly d(GC) · poly d(GC) in its A form (*a*), B form (*b*) and novel form (*c*). The specimens containing the A and B forms are also polycrystalline so that only Bragg reflections are observed. The symmetry of the new conformation is 226_5 and the space group of the diffracting structure probably $P226_5$. Which of the two molecular dyad axes (separated by $c/12$) is made to coincide with a space group dyad appears to be indeterminate. The result is a 'statistical' crystal structure[13] which gives both Bragg reflections and continuous streaks. The former are predicted to prevail on layer lines 0,2,5,7,9,14 the latter on layer lines 1,6,8,11,13 as observed.

us to be in no doubt that the new structure trapped in our oriented fibres is essentially the one that has been probed spectroscopically in 'high salt' solutions. The circumstances in which we have observed the transition to this new form in fibres supports this conclusion. It appears after prolonged annealing in some fibres prepared with the 3–6% retained NaCl necessary to reproduce the B conformation in typical DNA specimens. The left-handed helix has never appeared in fibres with minimum retained salt. With these, the A-DNA structure is observed initially and persists even after annealing for 30 months.

Apparently, left-handed helical conformations are not confined to poly d(GC) · poly d(GC) but are accessible certainly to other DNAs with alternating purine–pyrimidine nucleotide sequences. We have observed a similar diffraction pattern with one poly d(GT) · poly d(AC) specimen. This implies that pA can, when necessary, mimic the novel pG conformation. This conclusion is reinforced by the structure of poly d(As^{4}T) · poly d(As^{4}T). These molecules can have 7-fold screw symmetry[11] and pack in quasihexagonal unit cells with $a = b = 1.8$–2.3 nm and $c = 5.3$–5.4 nm depending on the relative humidity.

Although Saenger *et al.* interpreted the structure of poly d(As^{4}T) · poly d(As^{4}T) quite differently, we think that the diffraction patterns are so similar to Fig. 1*c* that a left-handed structure (Table 1) with $h = 0.76$ nm, $t = -51.4°$ (rather than 0.73 nm and −60°) provides a more plausible solution. Although s^{4}T nucleosides have been observed with the *syn* conformation in crystals[14,15], our modelling suggests that it is the purine (A) and not the pyrimidine (s^{4}T) nucleosides which play the conformationally unorthodox role like G nucleosides in the left-handed helix.

We have tested the possibility that our left-handed structure is accessible to DNAs with other base sequences by exchanging C and G and by imposing the homopolymer sequences of poly d(G) · poly d(C) on our model. Considerable steric compression results in both cases. Left-handed helical secondary structures that would accommodate any nucleotide sequence, therefore, would have to be somewhat different in conformational details.

Table 1 Comparison of conformation angles in the two common right-handed DNA helices with those of two left-handed helices

	A-DNA* (11_1, h=0.26 nm)	B-DNA† (10_1, h = 0.34 nm)	Poly d(GC) · poly d(GC)‡ (6_5, h = 0.73 nm)		Poly d(As^{4}T) · poly d(As^{4}T)§ (7_6, h = 0.76 nm)	
			CpG	GpC	s^{4}TpA	Aps^{4}T
θ(C4′–C3′–O3′–P)	175°	−133°	−72°	−103°	−92°	−106°
θ(C3′–O3′–P–O5′)	−45°	−157°	102°	−91°	99°	−77°
θ(O3′–P–O5′–C5′)	−90°	−41°	52°	−110°	60°	−96°
θ(P–O5′–C5′–C4′)	−149°	136°	−153°	−168°	−158°	−148°
θ(O5′–C5′–C4′–C3′)	47°	38°	178°	54°	161°	36°
θ(C5′–C4′–C3′–O3′)	83°	139°	76°	147°	80°	135°
θ(O4′–C1′–N9–C4)	−154°	−102°	89°	—	85°	—
θ(O4′–C1′–N1–C2)	−154°	−102°	—	−159°	—	−154°

Under each helix type are given the molecular screw symmetry and axial rise per asymmetric unit.

* From ref. 7 as adjusted for ref. 8.

† The C2′-*endo* model of ref. 9 re-refined by S.A. and R.C. (unpublished data).

‡ It is too early to claim that these are the only conformations consistent with the diffraction from poly d(GC) · poly d(GC) but they are similar to those of the core of d(CpG(pCpG)$_2$) in one of its crystallised duplex forms (G. J. Quigley, personal communication) and to the structure anticipated by Patel *et al.*[10] from their NMR study of d(CpG(pCpG)$_3$) in solutions of relatively high ionic strength (2.5 M NaCl). Certainly, the diffraction pattern predicted by the model (Figs 2, 3) closely resembles the one observed (Fig. 1*c*): the crystallographic residual for the Bragg reflections has a value $R = 0.27$.

§ This model for the structure of poly d(As^{4}T) · poly d(As^{4}T)[11] may not be the best solution.

DNA polymorphism

The considerable polymorphism of regular helical DNA duplexes has only recently been established. In the A genus, having polymononucleotides with C3′-*endo* furanose rings and all conformation angles of the same kind as A-DNA in Table 1, there is little variability in the rotation per nucleotide residue ($30° \leq t \leq 33°$) except for single-stranded molecules when $t = 60°$ is possible[16]. However the translation per residue (and therefore the mass per unit length) can vary by as much as 23% ($0.26\ \text{nm} \leq h \leq 0.33$) from A-DNA to poly d(T) · poly d(A) · poly d(T). In the B genus—polymononucleotides with C2′-*endo* furanose rings and all conformation angles as in B-DNA in Table 1—h is not quite so variable ($0.30 \leq h \leq 0.34$ nm) but t varies from its low value of 36° in B-DNA to its high value of 45° in D-DNA[17].

The discovery of a left-handed DNA conformation dramatically extends the range of secondary structures known to be allowed to DNA duplexes and may help to render moot many topological problems thought to be associated with some DNA activities. The known DNA conformations that are substantially overwound or underwound compared with B-DNA could be used to store or shunt locally the rotations needed to wind or unwind a double helix. For example, a segment of left-handed DNA, maintained in a region where the preferred sequences were sufficiently common, could be a store of negative windings. In appropriate conditions these could be used to compensate positive windings and produce a region of melted DNA available for polynucleotide synthesis. Conversely, regions of unwound DNA could condense to give contiguous segments of left-handed and right-handed helical DNA separated by as few as two nucleotides[18]. Such a structure might be a manageable substrate for topological isomerisation. Alternatively, the left-handed segment would form a package of negative windings which could be translocated easily even to a distant site.

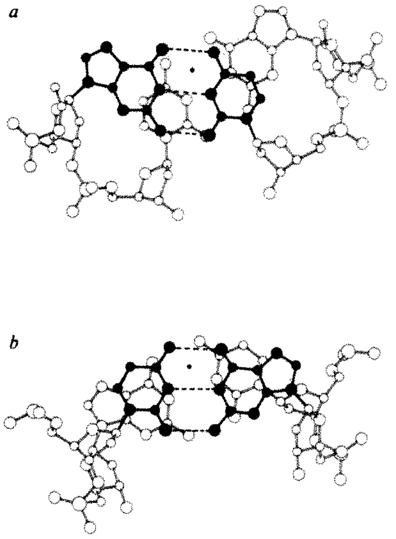

Fig. 2 The base stacking of *a*, GpC and *b*, CpG. The helix axis position is shown by a dot. A molecular dyad axis exists midway between the base pairs in each stack shown. In contrast to the more familiar polymononucleotide structures, the local dyad axis relating the glycosidic links of a base pair does not intersect the helix axis.

Overwound and underwound DNA secondary structures could also be associated with the creation or maintenance of supercoiled tertiary structures of either hand. When these are formed or destroyed, compensating changes in secondary structure are required. What these secondary structures might be has now been visualised.

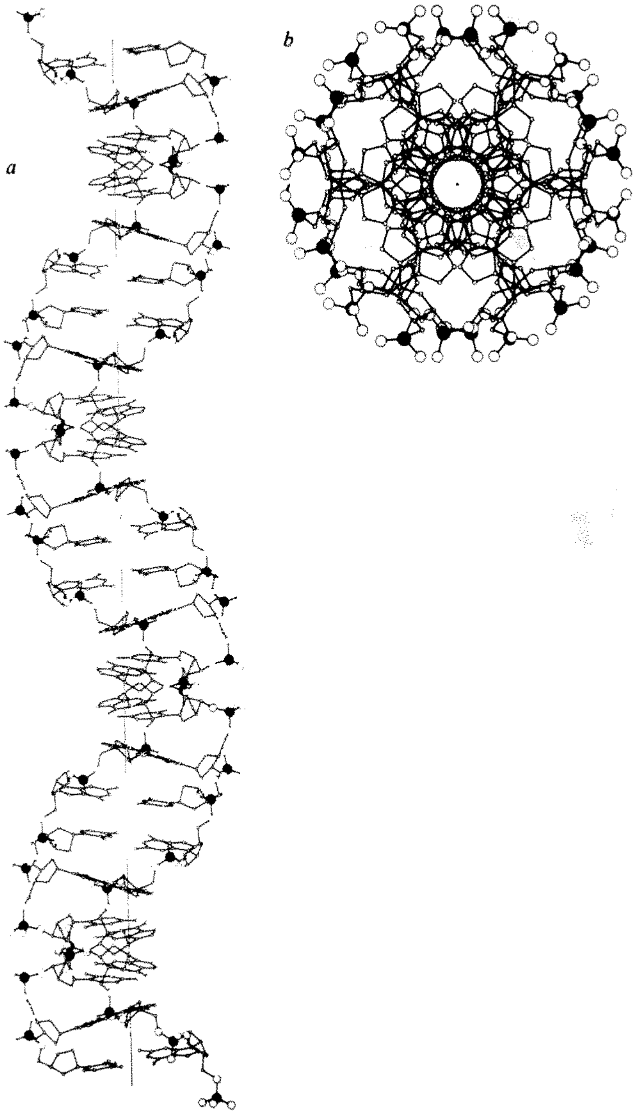

Fig. 3 A two-pitch length fragment of the new form of poly d(GC) · poly d(GC) viewed perpendicular to (*a*) and along (*b*) the helix axis and showing the disposition of the phosphate groups whose atoms are depicted with large circles.

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LETTERS

Is 0406 + 121 the reddest BL Lac object?

Marc Aaronson & Todd Boroson

Steward Observatory, University of Arizona, Tucson, Arizona 85721

0406 + 121 is a flat-spectrum variable radio source first discovered by Shimmins *et al.*[1] and later studied by Condon *et al.*[2]. While no optical counterpart was identified, similar sources were often found to be either BL Lac-type objects or high redshift QSOs. Recently, Rieke *et al.* detected the object in the near-IR, where it was observed to vary in luminosity over the six-month period between August 1978 and March 1979. A Kitt Peak 4-m plate taken on 29 November 1978 and published by Rieke *et al.* revealed a stellar image ($V = 20.5 \pm 0.4$ mag) at the radio (and IR) position, which lies ~30 arc s north of an anonymous spiral galaxy. Rieke *et al.* suggested that 0406 + 121 and some similar sources they detected having steep power law spectra were a possible new type of QSO discriminated against in conventional optical searches. To clarify the nature of 0406 + 121 further, we obtained a spectrum in good seeing conditions on the night of 27 February 1979 using the Steward Observatory 2.25-m telescope and intensified Reticon spectrograph. The object, clearly visible on an intensified TV guider, was wobbled every 80 s between two circular apertures 2.5 arc s in diameter spaced 20 arc s apart. Eighty-five minutes of integration time, calibration with the spectroscopic standard EG 67 (ref. 4) and a HeAr comparison lamp, and eight point smoothing of the 10 A resolution spectrum gave the results shown in Fig. 1.

The spectrum in Fig. 1 is consistent with a smoothly rising featureless continuum and seems to place 0406 + 121 in the class of BL Lac-type objects. However, the source is unusual in several respects. First, a $\nu^{-\alpha}$ power law fit to the data leads to $\alpha \approx 3.8$. The absorption implied at galactic latitude $b = 28°$ is $A_V \approx 0.11$ mag (ref. 5) so that corrected for reddening, $\alpha \approx 3.7$, to which we attach a conservative error of ±0.7. Taken at face value, the optical continuum of 0406 + 121 is thus the reddest known for any object in the BL Lac class. For comparison, α is ≈ 3.2 for AO0235 + 164 and ≈ 3.1 for BL Lac itself, the sources having the steepest previously known spectra[6].

A second curious property of 0406 + 121 concerns the possible lack of optical variability as compared with that seen in the near-IR. The V flux we derive from Fig. 1 is 20.2 ± 0.3 mag. This agrees closely with the photographic V magnitude measured three months earlier and quoted above. (A further check on this consistency was provided by our own visual estimate off the TV guider!) However, a systematic and substantial decrease in near-IR brightness was observed by Rieke *et al.*: they measured a K (2 μm) flux of ≈ 14.0 mag at the end of August 1978; a flux of ≈ 14.3 mag on 11 September 1978; a flux of ≈ 14.9 mag on 10 February 1979; and could not detect the object on 8 March 1979 (nine days after our spectrum was taken) when a 2 σ upper limit of ≈ 15.6 mag was obtained.

We have examined all of the published IR photometry of BL Lac sources compiled in Table 1 of Rieke and Lebofsky[7]. In every other instance when luminosity variations are observed, either the optical and near-IR flux change in step, or the optical flux changes over more rapid time scales. In particular, during periods of fading, the optical luminosity has been observed to decrease faster than in the near-IR in AO0235 + 164 (ref. 8) and in B21308 + 326 (ref. 9). If we interpret the Rieke *et al.* 2-μm observations as indicating a gradual and constant decline, then the optical and near-IR continua of 0406 + 121 are decoupled in a manner that is very atypical. This may have important implications in regard to the underlying source energetics (see ref. 10).

Unfortunately, neither of the V magnitudes (which are themselves more uncertain than desirable) were determined simultaneously with the IR data. Thus we cannot rule out the possibility, however unlikely, that our spectrum was taken during an outburst of 0406 + 121 (a common event for BL Lac objects) which then faded rapidly, giving the appearance of constant optical luminosity.

Weak evidence for at least some change in the optical flux of 0406 + 121 over the past 30 years is provided by the absence of the object on either the blue or red Palomar Observatory Sky Survey (POSS); for example, the present brightness above the red POSS plate limit is ≥ 0.5 mag.

In one respect 0406 + 121 does seem similar to its cousins AO0235 + 164 and BL Lac in that the near-IR spectral index seems less steep than in the optical. Physical reasons for such an effect have been considered by O'Dell *et al.*[8]. While again lack of simultaneously obtained data does not permit a conclusive decision, two indirect arguments do suggest the presence of a

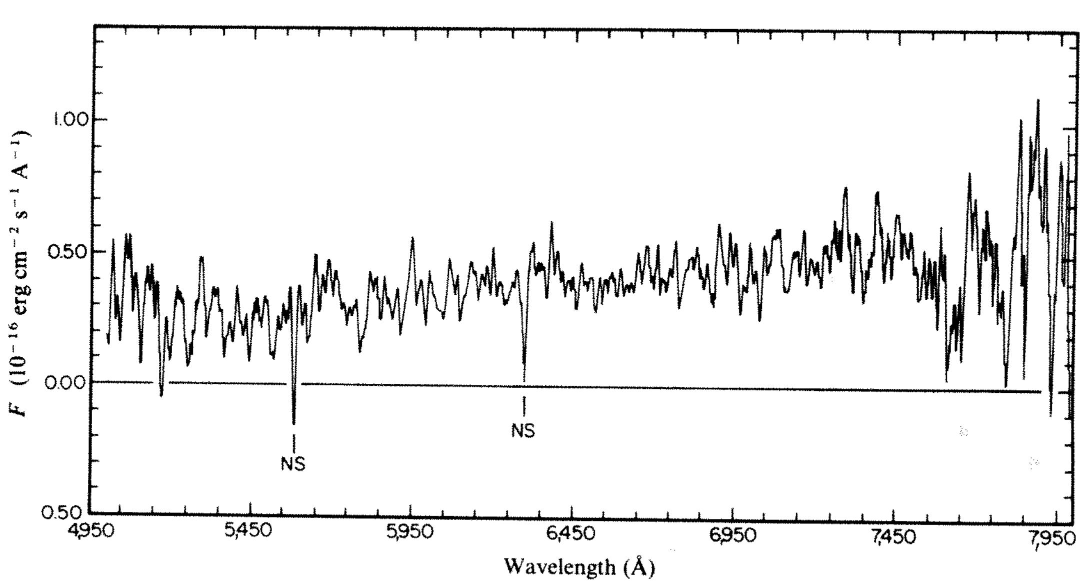

Fig. 1 A spectrum of 0406 + 121 is shown. Only a smoothly rising continuum is apparent, except for two poorly subtracted night sky lines. The spectrum becomes rather degraded shortward of 5,300 Å and longwards of 7,400 Å.

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spectral inflection point. First, the IR data of Rieke *et al.* are consistent with $\alpha \approx 2$–2.5, as compared with 3.7 here. Second, if our spectrum is extrapolated, then the predicted K flux is ≈ 10 times greater than a plausible interpolation of the 10 February and 8 March IR measurements. Any outburst this strong on such a short time scale would be unparalleled.

Clearly, further monitoring is essential to establish whether 0406 + 121 and similar objects are simply extreme cases of more familiar sources or represent a truly unique category.

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A dramatic radio outburst in the quasar 1921 – 29

William A. Dent & Thomas J. Balonek

Department of Physics and Astronomy, University of Massachusetts, Amherst, Massachusetts 01002

We report here an exceedingly large amplitude radio outburst presently occurring in the quasi-stellar source 1921 – 29 (also known as OV – 236). This source which had exhibited slow ±25% variations about a 6 Jy level (1 Jy = 10^{-26} W m^{-2} Hz^{-1}) at centimetre wavelengths since 1971 has suddenly increased by more than a factor of 3 to 20 Jy. At 31 GHz, 1921 – 29 is now the strongest known quasar in the sky, even exceeding 3C273 which has been decreasing since 1975.

Variation curves at four radio frequencies from 1971 to the present are shown in Fig. 1. These observations of 1921 – 29 are part of our study of the long-term spectral evolution of variable radio sources. The measurements at 89.6 and 31.4 GHz were made with the 36-foot antenna of the National Radio Astronomy Observatory[1,2] and those at 15.5 and 7.9 GHz were made using the 120-foot antenna of the Haystack Radio Observatory[3,4]. The sharp rise in flux density begins at 89.6 GHz near 1979.3 and is progressively delayed at lower frequencies. Radio spectra of the source at four different epochs are shown in Fig. 2. The spectral turnover below 15 GHz at 1979.5 and 1979.8 is characteristic of self-absorption in a source emitting synchrotron radiation. The broad spectral maxima imply an inhomogeneous distribution of radiating particles and magnetic field.

The recent outburst is itself superimposed on a more slowly varying component which increased gradually through 1976 and 1977 and then remained constant until early 1979. Its broad flat spectrum in Fig. 2 is also characteristic of a self-absorbed inhomogeneous source. The high frequency transparent portion of the spectrum has a slope of $\alpha \approx -0.4$ above 31 GHz. Like the current 1979 outburst, the rise during 1977 of this slow component was self-absorbed and delayed at lower frequencies.

The spectrum of the rapid 1979 outburst at different epochs can be obtained (Fig. 3) by subtracting the spectrum of the source in 1978, just before the recent outburst. We see that the outburst itself has an initially steep low frequency cut-off with spectral index $\alpha \approx +2$ below 15 GHz. As the outburst evolves its low frequency spectrum flattens as the frequency of maximum decreases from about 100 to 30 GHz. The high frequency spectrum above 31 GHz becomes transparent with $\alpha \approx -0.3$, implying $\gamma = 1 - 2\alpha = 1.6$ in a relativistic electron distribution of the form $N(E)\,dE \propto E^{-\gamma}\,dE$. Such a hard electron distribution is characteristic of most variable radio sources. This distribution seems to be preserved over the different phase of activity in the source demonstrating the consistency of the particle acceleration mechanism.

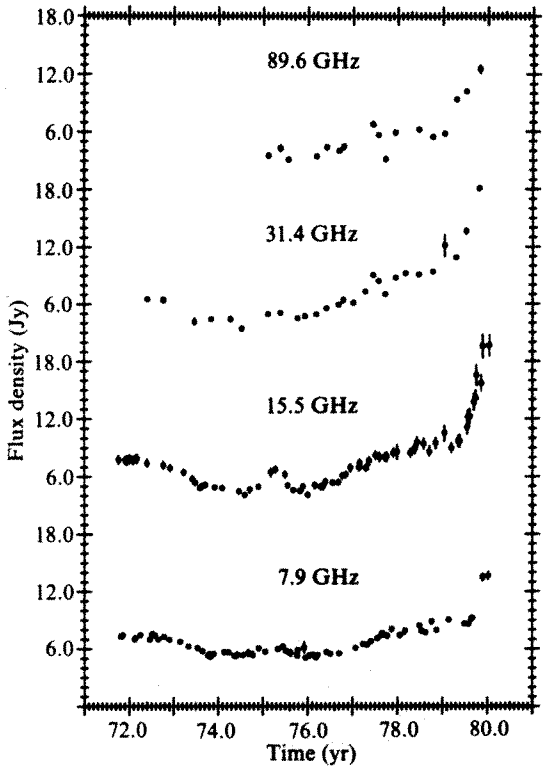

Fig. 1 The observed variation of 1921 – 29 at four radio frequencies.

The spectral evolution of both the slow 1977 outburst and the rapid 1979 outburst suggest that both are expanding from an initially smaller volume[5]. The initially sharp and eventually broad low frequency turnover of the slow outburst shows that an initially homogeneous component became inhomogeneous as it expanded. The pronounced difference in time scales of the two events must be due either to different rates of expansion or to time compression if there is relativistic motion near the line of sight.

Even though it does not have a published redshift, 1921 – 29 is undoubtedly a quasar. It has been identified as a stellar object

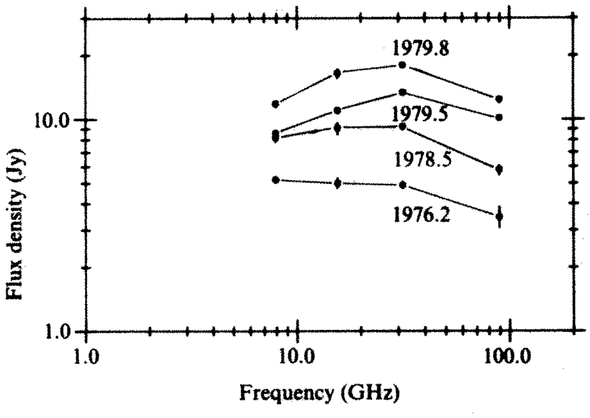

Fig. 2 The total radio spectrum of 1921 – 29 at four epochs.

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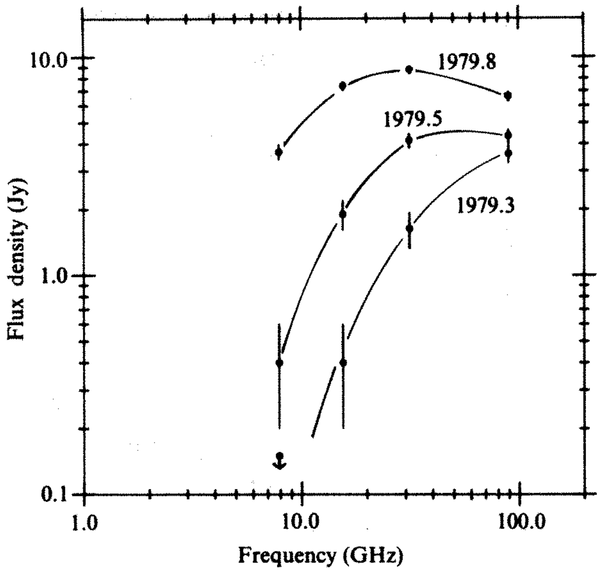

Fig. 3 The spectrum of the 1979 outburst at three epochs obtained by subtracting the average 1978 radio spectrum during 1978.

with a red colour[6] and flat radio spectrum[7]; it is variable at both radio and optical frequencies[8] and has angular structure $<10^{-3}$ arc s at 13 cm (ref. 9). The optical position of this 17 mag quasar is 19 h 21 min 41.42 s and −29°20′25.3″. Optical and X-ray observations of its spectrum and variability during the current outburst would be very valuable.

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Thermal history, chemical composition and relationship of comets to the origin of life

W. M. Irvine*†, S. B. Leschine‡ & F. P. Schloerb*

* Department of Physics and Astronomy, University of Massachusetts, and † Onsala Space Observatory, Chalmers University of Technology, Sweden
‡ Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

It is generally believed that a comet consists basically of a loose conglomeration of frozen gases with embedded material similar to that found in the carbonaceous chondritic meteorites, and consequently that comets may be nearly pristine samples of the original solar nebula[1–5]. We show here that thermal processing within comets could have played an important part in determining their present state; in particular, we find that liquid water might have been available in some comets over geologically and biologically significant spans of time. It follows that a cometary origin is not excluded for some thermally metamorphosed meteorites and asteroids, that comets may contain quite complex organic molecules, and that comets may have played a role in the origin and conceivably even in the subsequent evolution of terrestrial life.

Given a mixture of ices and chondritic material with the normal abundance of radioactive elements (U, Th, and K), Lewis[6] and Whipple and Stefanik[7] have shown that the central temperature in comets will not exceed the melting point of H_2O. Recently, however, inclusions with anomalous isotopic abundances have been found in certain meteorites which suggest that a nearby supernova explosion may have injected significant amounts of the short-lived radionuclide ^{26}Al into the condensing solar nebula[8]. In fact, the necessity for such a short-lived heat source was earlier implied from studies of the mineralogy of meteorites[9,10]. As it seems likely that comets formed in the outer portion of the Solar System, at least some of these objects may have received a particularly large dose of such 'extra-solar' material. An important parameter affecting the subsequent thermal history of a comet is the size of the cometary nucleus. Direct observations are largely non-existent, but present evidence is consistent with a model in which at least some new comets have radii of the order of 10 km (refs 11–14), while occasional cometary nuclei could be an order of magnitude or more larger (the largest presently observable possibly cometary object may be Chiron[15], whose radius is probably ~100 km (E. Bowell, personal communication)).

Will the surface temperature of a comet heated by such short-lived radioactivity be cool enough to retain the volatile material observed? An upper limit to the surface temperature can be obtained by assuming a sufficiently high thermal conductivity that all internally generated heat is immediately radiated away. The abundance of ^{26}Al in the meteoritic inclusions[8,16] is $^{26}\mathrm{Al}/^{27}\mathrm{Al} = {\sim}5\times10^{-5}$, which yields an available heat of 2 kcal g^{-1}. Since the half-life of ^{26}Al is 7×10^5 yr, we find that heat is generated at a rate $Q \simeq 2\times10^{-3}$ erg g^{-1} s^{-1}. The corresponding surface temperature T_s for a cometary nucleus of density ρ and radius R is $T_s \leqslant (QR\rho/3\sigma)^{1/4} \leqslant 120$ K for $R \leqslant$ 100 km, $\rho \simeq 2$ g cm^{-3}, and σ = the Stefan–Boltzmann constant. As this is an upper limit and T_s will decrease as the heat source decays, H_2O is certainly retained by the comet[17]; and H_2O may control the vaporisation of more volatile compounds, if these are trapped as clathrates in the H_2O lattice[18].

The temperature is potentially quite different in the interior of a comet. The abundance of ^{26}Al is sufficient to melt silicates, let alone icy material, provided thermal loss to the surface of the body is sufficiently slow[16] and provided (as we here assume) that the formation time of comets is not long compared with the ^{26}Al half life. Thus, even if significantly diluted by material without such short-lived radionuclides, sufficient energy would be available to raise the interior temperature of a comet to several hundred degrees. The time scale t_{int} for cooling the central regions is approximately $t_{\mathrm{int}} \simeq R^2\rho C_p/k = R^2/\kappa$ where k is the thermal conductivity and $\kappa = k/\rho C_p$ is the thermal diffusivity. At $T = 120$ K (appropriate to some intermediate region in the mantle of the comet) the thermal constants for ice are $C_p = 10^7$ and $k = 6\times10^5$ in c.g.s. units[19,20], so that the thermal diffusivity is $\kappa \simeq 3\times10^{-2}$ cm^2 s^{-1} for $\rho \simeq 2$ g cm^{-3}. For a loosely compacted body, however, κ might be significantly lower; for the Moon κ varies from $\simeq10^{-4}$ cm^2 s^{-1} near the surface to $\simeq10^{-3}$ cm^2 s^{-1} in the deeper regolith[21,22]. Counteracting the latter effect might be the possibility of thermal transport by gaseous diffusion and convection. If we take the intermediate value $\kappa = 0.004$ cm^2 s^{-1}, we find $t_{\mathrm{int}} \simeq 10^9$ yr for $R = 100$ km. For the more typical radius 10 km for a new comet, we find a cooling time for the interior of the order of 10^7 yr. These times might in fact be significantly lengthened by the accretion of an insulating dust layer, as Wood has proposed for the parent planetesimals of certain meteorite classes[23].

Note the following astronomical consequences. Some comets may have undergone significant thermal processing, so that the apparent exposure of, for example, chondritic meteorites and probably some Apollo asteroids to temperatures of several hundred degrees[24] does not in itself exclude their being derived from comets. Such an origin has been supported on dynamical[25] and spectroscopic[26] grounds, although other problems remain[27]. (If, instead of being initially homogeneous, comets were formed by accretion of volatiles onto an asteroid core[28,29], a similar generic relationship could exist.) Likewise, the existence in new comets of a particularly volatile outer shell, suggested by the appearance of a gaseous coma at large distances from the Sun[30], is a natural consequence, although this does not require as strong a heat source as considered here[7]. The possibility of thermal processing must be considered in calculating the chemical composition of the cometary volatiles. The original composition of the nucleus, like that of the Sun and the interstellar medium, will be reducing. Heating such a mixture, particularly in the presence of the metallic and clay catalysts expected to be present, results in the production of organic molecules, including amino acids and nucleic acid bases[31–33]. Complex organic molecules are also produced in such mixtures by ionising radiation, which would result from the radioactive decay postulated here[34].

Could comets be relevant to the origin of life? A principal problem in the synthesis of biologically significant molecules in the aqueous terrestrial environment is the polymerisation of amino acids and nucleic acid monomers. Dehydration reactions involving volcanic heating and/or evaporation and catalysis in tidal pools have been invoked[32,35]. Suitable environments might more naturally occur in a comet, where we may suppose temperature regimes from considerably above the boiling point of H_2O to a perhaps substantially depressed (by dissolved salts) freezing point, solid clay and metallic surfaces for catalysis, possible circulation of an aqueous solution with dissolved organics, and much less dilution than would be expected in a terrestrial ocean. Moreover, the time span determined above during which liquid water might be available in some comets is consistent with geochemical evidence for the epoch of the first O_2-releasing photosynthesis on Earth[36]. The biological requirement for free energy in a dark environment has been considered by Anders[37] in a discussion, similar to the above, concerning the possibility that asteroids might have supported life (we note also the existence of apparently photosynthesis-independent biocommunities at mid-ocean thermal vents[38]). Present models postulate that the Sun is surrounded by a vast cloud containing perhaps 10^{11}–10^{12} comets[39] and thus providing environments for a vast number of 'biological experiments'. When a comet enters the inner Solar System, it leaves behind a trail of debris which is encountered by the Earth as meteor showers and micrometeoroids. The Earth accretes about 10^7 kg yr^{-1} of such material[5,40]. Micrometeoroids smaller than 10^{-6} g will not be heated above 100 °C on entry, and the interiors of meteorite-sized objects also remain at moderate temperatures[41,42]. It follows that comets may have played a part in seeding the primitive Earth with biological polymers possibly capable of self-replication, or at least of evolving towards that capability.

Is it conceivable, as suggested by Hoyle and Wickramasinghe[43,44], that in subsequent times the Earth has been infected by organic material which arose independently in cometary environments and which was viable in terrestrial organisms? The resemblance between the genetic systems of viruses and cells implies that the evolution of viruses depends on the evolution of cellular genetic systems[45] and therefore makes the independent evolution of viruses on many different comets very unlikely. On the other hand, it seems difficult to exclude the possibility that small nucleic acids of cometary origin might occasionally be viable in terrestrial organisms if (in the apparent manner of viroids[46,47]) they could reproduce without the expression of self-specified proteins, so that they need not have evolved a genetic code translatable by the host cell.

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Neutron holography using Fresnel zone plate encoding

T. D. Beynon & A. G. Pink

Department of Physics, University of Birmingham,
Birmingham B15 2TT, UK

Zone plate shadow casting is a complementary technique to the Leith–Upatnieks modification of Gabor's interferometric method for producing holograms[1,2]. Suggested originally by Mertz[3] in 1961 for use in X-ray astronomy, the technique has received considerable attention in nuclear medicine[4] for imaging radioactive organs via emitted X rays and γ-rays and, more recently, for examining in a tomographic sense the microscopic spatial source distributions of X rays and charged particles in laser-produced plasmas[5]. A series of simple experiments are described here which demonstrate that zone plate encoded neutron holography can be used to image objects. An object placed in a cold neutron beam from a research reactor scatters neutrons through a Fresnel zone plate, with alternate zones of gadolinium and aluminium, which produces a hologram of the object on an X-ray film. The image is constructed by placing the hologram, linearly reduced in size, in a converging laser beam which produces a real image of the original object. Results are presented for two objects, a pair of 0.01 m diameter rubber spheres and a 0.04 m square Maltese cross made from polytetrafluoroethylene (PTFE).

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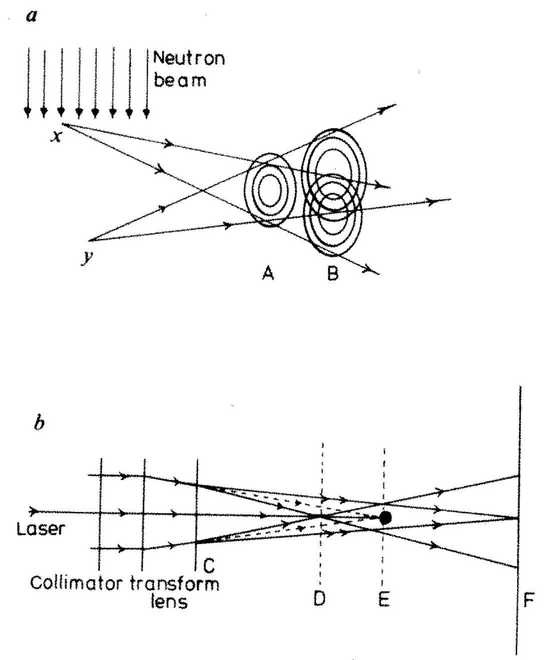

Fig. 1 *a*, Schematic arrangement of two point sources at x and y producing, through a fresnel zone plate at A, a zone plate hologram in a plane at B. The direction of the neutron beam is parallel to the zone plate at A. *b*, The optical arrangement for the reconstruction at E of the object encoded in the hologram at C.

The basic principles of zone plate encoded holography are outlined in Fig. 1. If we consider (Fig. 1*a*) two point sources of radiation at x and y, a Fresnel zone plate at position A will produce in a plane at B two overlapping zone plate shadowgraphs, provided that the wavelength of the radiation from x and y is sufficiently small not to be diffracted by the zone plate. Any solid object, regarded as a linear superposition of points, will produce on a suitable recording material in a plane B a two dimensional hologram consisting of a linear superposition of zone plate shadowgraphs in which is encoded non-coherently the full three dimensional information of the object. The reconstruction of the original object may be achieved coherently by using the optical properties of a Fresnel zone plate, as shown in Fig. 1*b*, which illustrates what is essentially a hardware analogue Fourier transform of the hologram. Each single zone plate in the hologram, acting as a lens, will produce a unique point image of the object point which produced the shadowgraph zone plate. A converging coherent light beam, produced by the transform lens, passes through a linearly reduced transparency of the hologram at a position C. The undiffracted component of the beam is stopped at E. The image at D, due to the negative focal length of the hologram, is superimposed as background on the desired image at E which is due to the hologram's positive focal length. The image in a particular plane through F is the reconstruction of a particular plane in the original object parallel to the original zone plate (Fig. 1*a*). All such object planes may now be retrieved by recording the images on the F-planes spanning the space in which the real image appears.

Neutron holograms have been made for several objects using the 6H beam tube on the DIDO research reactor at AERE, Harwell. This facility provides a well-collimated beam of cold neutrons (energies < 5 meV) with a very low γ-ray contamination. A description of this facility is given elsewhere[6]. As it is those neutrons which are scattered off the object into the zone plate which form the hologram, as illustrated in Fig. 1*a*, it is necessary to work in as large a neutron flux as possible. Consequently all the experiments were carried out inside the reactor shell wall at a distance of 2 m from the reactor face where foil activation measurements gave a value for the neutron flux strength of 3.3×10^{11} neutrons $\mathrm{m}^{-2}\mathrm{s}^{-1}$.

The Fresnel zone plate was made by photographically etching a zone plate pattern ($r_n = r_1\sqrt{n}$, $n = 1, 2, 3, \ldots$) onto a 1.2-mm thick aluminium alloy sheet to a depth of 100 μm and filling the etched zones with a paint containing gadolinium. The aluminium has a low scattering cross-section and is more than 99% transparent to cold neutrons, whilst the gadolinium is essentially opaque. The plate had 47 zones with a first-zone radius (r_1) of 1.12×10^{-2} m. The holograms were recorded on 0.18 m × 0.24 m X-ray films with a gadolinium foil intensifying screen in front of the film to ensure a linear response in the film emulsion during the relatively long exposure times at these low neutron flux levels. With the recording film 0.4 m from the zone plate which was placed typically at 0.15 m from the objects, with its

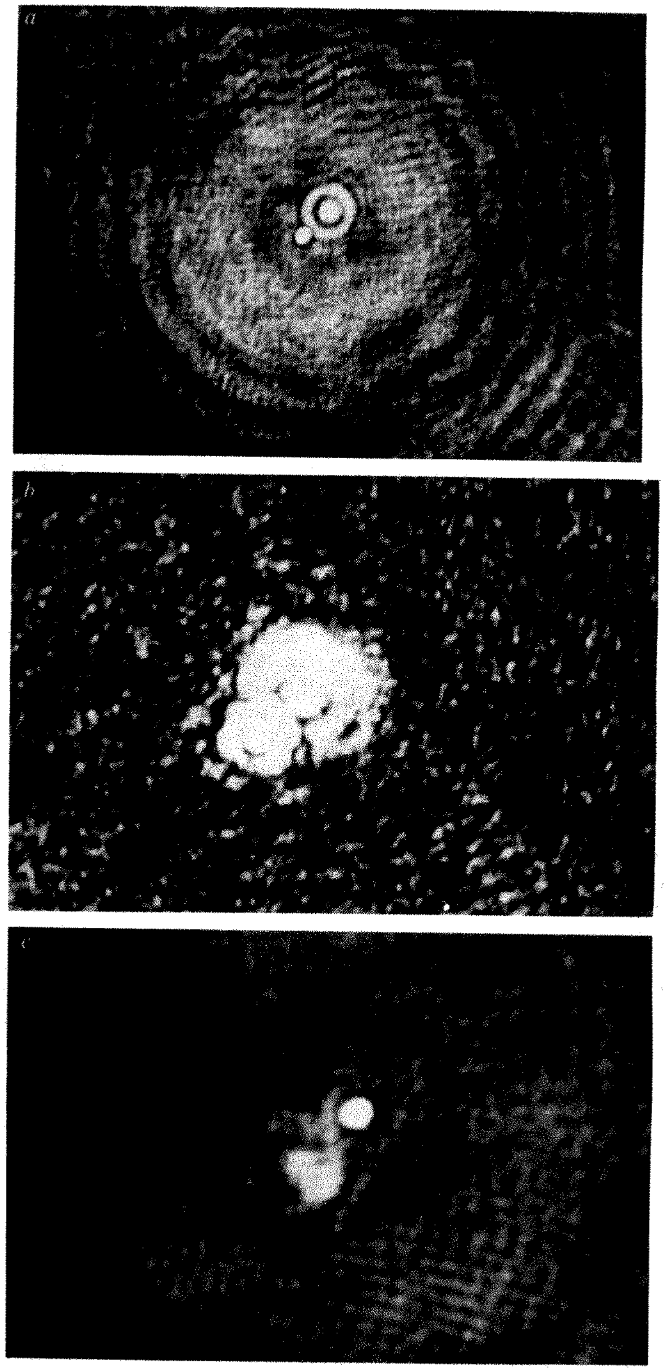

Fig. 2 *a*, A plane through one reconstructed image of sphere (lower left), the other appearing out of focus. *b*, A plane between the spherical images, intersecting neither. *c*, A plane through the second reconstructed image of sphere (upper right), the first image now appearing out of focus.

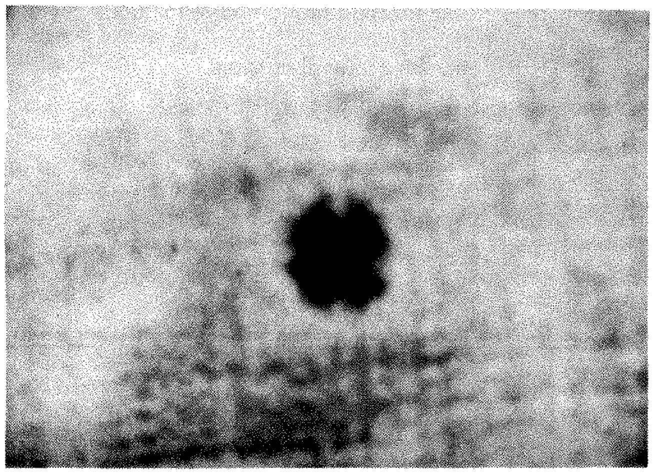

Fig. 3 Optical reconstruction of the Maltese cross.

plane parallel to the beam direction, exposure times using Kodak Industrex C film were 1.5–2 h.

The optical reconstruction was achieved with the arrangement shown in Fig. 1*b*, with a 2 mW helium–neon laser and a transform lens of focal length 0.15 m. Each neutron hologram was linearly reduced in size by a factor of 40 and a conventional transparency made using Agfa-Gevaert high resolution holographic film type 10E56. Note that a bleaching process recommended for this film, which forms the image by having a desensitised silver halide residue rather than silver grains, can produce reconstructed images with considerably enhanced intensity.

We illustrate the effectiveness of the technique using results for two encoded objects of markedly different geometry. The first test object consisted of two rubber spheres, diameter 0.01 m, suspended in the neutron beam with a horizontal and vertical separation of 0.04 m. The second object was a Maltese cross cut from a 0.04 m square of PTFE of thickness 4 mm. The cross was placed with its plane at an angle of 10° to the plane of the zone plate. A series of planes through the reconstructed images have been recorded on standard fine grain photographic film. Figure 2 shows three such planes through the spatially-separated spherical images of the two original spheres. Figure 2*a* is a plane through the first sphere at a distance of one-half radius from the surface; the second sphere appears out of focus. Figure 2*b* is a plane midway between the spherical images and intersecting neither; both images appear out of focus. Figure 2*c* is a plane through the centre of the second spherical image, the first now appearing out of focus. Finally in Fig. 3 we show a plane through the reconstructed image of our PTFE Maltese cross. Figures 2 and 3 are based on image sizes of about 1 mm. Practical applications of this technique would require larger image sizes which could be achieved by using a telescope in the optical reconstruction system of Fig. 1*b*. A more detailed technical discussion of this work, including a description of computer-aided reconstructions of the geometry of the images and an assessment of the limits of resolution of the technique, will be published elsewhere.

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Why noble metals favour the face-centred cubic structure

C. S. G. Cousins

Physics Department, Exeter University, Exeter EX4 4QL, UK

The noble metals (copper, silver and gold) crystallise in the face-centred cubic (f.c.c.) structure but attempts to calculate their cohesive energy[1] and stacking fault energies[2] predict that the stable structure is the hexagonal close packed (h.c.p.). By considering a collinear three-ion interaction I have identified a source of energy, of the correct sign and order of magnitude, that explains the stability of the observed structure. Its success is largely due to a distinctive difference in number and spatial disposition of close-packed lines of atoms in the two structures. The principal parameters of the calculation are the scattering phase shifts of the ions and it is therefore highly probable that three-ion interactions also play a significant part in the stability of the transition metals.

The contributions usually considered in studies of metallic cohesion and stability are the Ewald energy, the core overlap energy, the kinetic, exchange and correlation energies of free electrons, and a band-structure energy that is calculated to second order in perturbation theory. Applying such an approach to the noble metals shows that of the two close-packed structures, the hexagonal one is to be preferred because $\Delta E \equiv E_{\mathrm{fcc}} - E_{\mathrm{hcp}} \approx 0.01$ to 0.003 eV per atom (ref. 1). A related study of stacking fault energies[2], whose principal considerations are the structure-dependent terms in the energy, is also unsuccessful: it predicts positive energies for the h.c.p. structure and negative ones for the f.c.c. Although minor adjustments or refinements to the usual theory might reverse the sign of the calculated ΔE, I believe that the f.c.c. stability is caused by three-ion interactions and that only these can provide the substantial $\Delta E \approx -0.01$ to -0.03 eV per atom needed to explain the measured stacking fault energies.

This idea is supported by Krause[3]; he examined a subtle effect in the lattice dynamics of copper and showed that an interplanar stiffness constant derived from $\langle 111 \rangle T$ phonon dispersion data was incompatible with an exclusively two-body potential.

In a study of multi-ion interactions[4] in simple metals Harrison has drawn attention to a three-ion term in which the ions are collinear and separated by nearest neighbour distances. This is a dominant term because it involves the matrix element for forward scattering and therefore mimics a second order term in

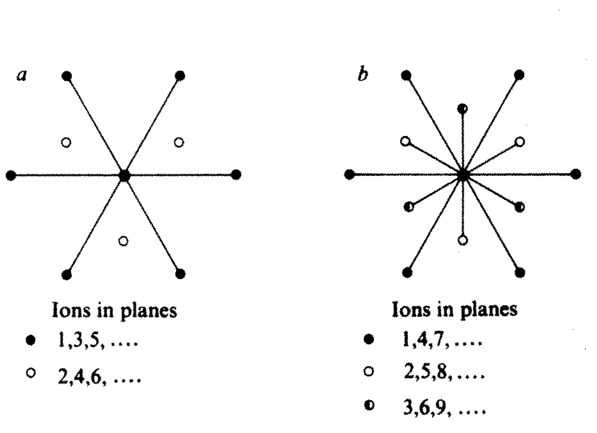

Fig. 1 The disposition of linear triads of ions in the h.c.p. and f.c.c. structures. The projections of ion positions are shown looking down; *a*, the [00.1] axis in the h.c.p. structure; and *b*, the [111] axis in the f.c.c. structure.

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Table 1 The contribution of the dominant three-ion term to the energy difference between f.c.c. and h.c.p. forms of the noble metals

	Copper			Silver	Gold
E_F* (eV)	6.80	7.48	8.16	5.58	5.58
$\delta(\pi)$	0.009	−0.027	−0.076	−0.072	−0.265
$\delta(0)$	0.748	2.418	2.969	1.260	1.109
ψ	0.766	2.364	2.817	1.116	0.580
$\lvert t(2k_F)\rvert$ (eV)	2.30	2.84	3.41	1.55	1.97
V_0 (eV)	22.1	30.5	40.3	12.2	19.7
Φ_3 (eV per triad)	−0.007	−0.018	−0.015	−0.006	−0.010
ΔE_3 (eV per atom)	−0.022	−0.053	−0.044	−0.019	−0.030

* The free electron values of E_F are 7.00, 5.48 and 5.51 eV respectively.

magnitude. To avoid a complex analysis only this term will be discussed below.

Each atom in an h.c.p. structure is the central atom of three triads which are confined to the close-packed plane (Fig. 1). Each atom in an f.c.c. structure is the central atom of six triads, three lying in the close-packed plane and three spanning three such planes. The net result is a contribution to the energy of the f.c.c. structure that differs from that of the h.c.p. structure by $\Delta E_3 = 3\Phi_3$ where the energy of a single triad is given by

$$\Phi_3 = V_0 8\pi Z \cos(4k_F d + \psi)/(4k_F d)^4 \tag{1}$$

This expression, derived from Lloyd and Oglesby[5], departs from Harrison's pseudopotential treatment in favour of a form based on scattering phase shifts and, therefore, more appropriate to the noble metals. d is the nearest neighbour distance and $(4k_F d)^3 = 192\sqrt{2}\pi^2 Z$, where Z is the valence (unity in the calculation that follows) and $V_0 = 9\pi Z^2|t(2k_F|^2/E_F$ where $t(2k_F)$ is the t-matrix for on-Fermi-surface back scattering and E_F is the Fermi energy. The phase ψ and $|t(2k_F)|$ are derived from the η_l, the scattering phase shifts for an ion evaluated at the Fermi energy, and are given by

$$\psi = 2\delta(\pi) + \delta(0)$$

and

$$|t(2k_F)| = 4E_F|(\alpha(\pi))^2 + (\beta(\pi))^2|^{1/2}/3\pi Z$$

where $\cot \delta(\theta) = \alpha(\theta)/\beta(\theta)$,

$$\alpha(\theta) = \Sigma_l (2l+1) \sin \eta_l \cos \eta_l P_l(\cos\theta)$$

and

$$\beta(\theta) = \Sigma_l (2l+1) \sin^2 \eta_l P_l(\cos\theta)$$

In the limit of small perturbation $|t(2k_F)| \to v(2k_F)$ and $\delta(\pi) \to 0$. Because the perturbation is not small, $|t(2k_F)|/E_F > 0.28$ in the present case, the phase shift method is being used. The data required for the evaluation of equation (1) can be found elsewhere[6,7] in work in which a precise parameterisation of the Fermi surface is given in terms of four phase shifts. Here the Fermi energy is a free parameter and three sets of results for copper have been given[6]. Table 1 contains the parameters derived from these works and the resulting ΔE_3, which ranges from −0.02 to −0.05 eV per atom, and may be compared with the experimental estimates[8] of the intrinsic stacking fault energy: 0.019, 0.010 and 0.022 eV per atom in the fault plane for Cu, Ag and Au respectively. As the contribution of three-ion interactions to the intrinsic stacking fault energy is $-2\Delta E_3$ (ref. 9) it is clear that while these results are in the correct sequence they are, nevertheless, too large. Some reduction in the value of ΔE_3 is to be expected in a full three-ion calculation but a change of sign is highly unlikely. This is because the numbers of the smallest triangular configurations are very similar in f.c.c. and h.c.p. structures. Both have 24 equilateral configurations and 12 configurations with sides in the ratio $1:1:\sqrt{2}$, and neither of these sets can provide an energy difference between the structures. I conclude therefore, that three-ion interactions are exclusively responsible for the stabilisation of the noble metals in the f.c.c. structure.

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^{15}N natural abundance in oceanic suspended particulate matter

Toshiro Saino & Akihiko Hattori

Ocean Research Institute, University of Tokyo, Nakano-ku,
Tokyo 164, Japan

Particulate organic matter (POM) has a central role in the vertical transport of material in the sea[1]. In the open ocean, POM is produced in the euphotic layer by phytoplankton and degraded in the aphotic layer during sinking to the sea floor. Isotopic abundance of biophilic elements such as C and N in POM is altered by isotopic fractionations associated with biochemical reactions[2]. Natural abundances of ^{13}C or ^{15}N thus provide useful information on biochemical behaviour of POM in the sea. We report here the first comprehensive data on the vertical distribution of ^{15}N in suspended POM[3] collected at a station in the northeastern Indian Ocean. Also, we discuss how nitrogen isotopic analysis could be used for the identification and quantification of the vertical transport processes.

Williams and Gordon[4] have investigated ^{13}C abundances in suspended particulate organic carbon (POC) collected from the eastern North Pacific, between 10 and 2,940 m, the δ^{13}C values fell in the narrow range −22.0 to −24.3 ‰. They suggested that most of the suspended POC is biologically refractory. A variation of δ^{13}C in POC with depth was later demonstrated by Eadie and Jeffrey[5]; POC in deeper layers (>330 m) was significantly lower in ^{13}C than that in shallower layers. C/N ratios of suspended POM increase with depth[6]. This implies that nitrogen is more rapidly lost than carbon during degradation of suspended POM. Therefore, a much greater variation of ^{15}N abundance in suspended particulate organic nitrogen (PON) is expected than in ^{13}C in POC.

Seawater samples were collected with Van Dorn bottles (25 l) from various depths or with a plastic bucket from the sea surface on Cruise KH-76-5 of Hakuho Maru, University of Tokyo[7]. 20–23 l of seawater was filtered through a precombusted (400 °C, for 1 h) Reeve Angel 984H glass fibre filter (47 mm diameter). The POM collected on the filters was desiccated over silica gel *in vacuo*. The procedures for conversion of PON to nitrogen gas and its purification have been described elsewhere[8]. The total nitrogen and its ^{15}N content were determined simultaneously using a Hitachi RMU-6E mass spectrometer fitted with a double collector and a dual inlet system. Sufficient nitrogen gas was introduced into the mass spectrometer to produce an output signal at $m/e = 28$ of greater than 7 V. Isotopic ratio measurements were repeated at least four times for each sample and averaged. Two sources of reagent grade ammonium sulphate (δ^{15}N; −6.7 and −1.5‰ relative to atmosheric nitrogen) were used as isotopic standards. Reproducibility of each measurement was ±0.3‰.

Table 1 summarises the δ^{15}N values of duplicate samples collected from the sea surface at 12 different locations. Differences in the δ^{15}N of the duplicate determinations ranged

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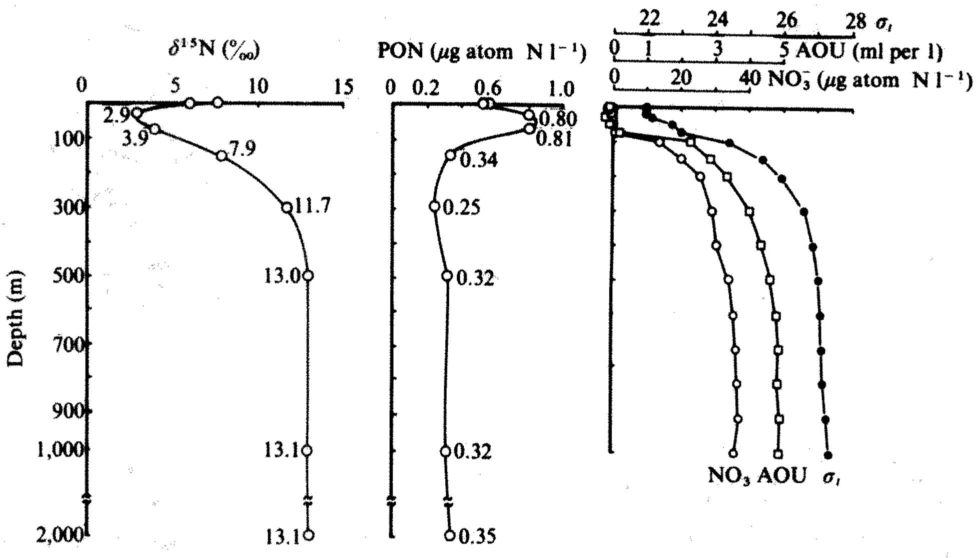

Fig. 1 Vertical distribution of $\delta^{15}N$, PON, σ_t, AOU and NO_3^- at Station 5 in the northeastern Indian Ocean on 11 January, 1977. Water samples for temperature, salinity, dissolved oxygen and nitrate determinations were collected with Nansen bottles equipped with reversing thermometers. Nitrate was measured using a Technicon Autoanalyser II on board the ship. σ_t and AOU are cited from the cruise report[7].

from 0.3 to 2.5‰, averaged 1.4‰. Heterogeneity in the distribution of POM and in its isotopic composition may partly cause this variation[8].

Figure 1 presents the vertical distribution of $\delta^{15}N$ along with PON, σ_t, apparent oxygen utilisation (AOU)[9] and nitrate at a station located in the northeastern Indian Ocean. $\delta^{15}N$ decreased near the surface reaching a 2.9‰ minimum at 30 m and then increased through the 30–500 m depth by ~10‰. Below 500 m the $\delta^{15}N$ remained constant (~13‰). Conversely, PON increased near the surface and attained a maximum between 30 and 75 m. The abundance of ^{15}N in marine organisms has been shown to increase with increasing trophic level[10]. The $\delta^{15}N$ values for PON collected from the euphotic layer (<150 m), where production of POM exceeds its degradation, are similar to those for phytoplankton while the $\delta^{15}N$ values for PON from depths >300 m are similar to those of zooplankton or fish.

The increase of $\delta^{15}N$ with depth parallels the increase in σ_t, AOU and nitrate. This suggests that the ^{15}N enrichment in PON with depth results from isotopic fractionation during oxidative degradation of PON. Wada[11] has found that $\delta^{15}N$ increases by 3–4‰ during the early period of oxidative degradation of dead phytoplankton and zooplankton at room temperature and atmospheric pressure. Our results suggest that degradation of PON actively proceeds in the upper 500 m, leaving refractory components which sink into the deep layers.

Low values for $\delta^{15}N$ in the subsurace PON may result from isotopic fractionation associated with nitrate uptake by phytoplankton in light-limited conditions, because growth of phytoplankton in this subsurface layer is mainly supported by nitrate[12]. Preferential utilisation of ^{14}N-nitrate over ^{15}N-nitrate by marine diatoms has been reported[13]. The ^{15}N enrichment of nitrate in eastern Pacific shallow waters over that in deeper waters also supports this view[14].

In the surface layer of the tropical Indian Ocean, the concentrations of nitrate are extremely low, and the growth of phytoplankton is mainly sustained by regenerated ammonia[15]. Under these circumstances, isotopic fractionation is minimal and the relatively high $\delta^{15}N$ values for surface PON can be attributed to a rapid recycling of nitrogen[16].

There are two main mechanisms for the vertical transport of particulate matter in the sea: (1) transportation mediated by slowly sinking fine suspended particles, which are easily collected on filters by filtering small amounts of seawater; and (2) transportation mediated by rapidly sinking larger particles such as faecal material, which are not collected adequately by conventional water sampling[3], but can be collected by *in situ* filtration of large seawater volumes[17] or by use of sediment traps[18,19].

The $\delta^{15}N$ values reported for organic nitrogen in marine sediments are 10‰ or less[10,20]. The $\delta^{15}N$ values for suspended PON in deep water is much higher (Fig. 1). If the organic nitrogen in sediments is solely supplied by suspended PON, the lower sediment organic nitrogen $\delta^{15}N$ values are difficult to explain. A recent survey that we conducted in the northern North Pacific found that PON collected with sediment traps is invariably less enriched with ^{15}N ($\delta^{15}N$, 2.9–4.4‰) than are sediments, irrespective of the collection depth (1.1–5.25 km). Simultaneous determinations of ^{15}N abundance in suspended PON, settling PON collected with sediment traps, and sedimentary organic nitrogen should provide an estimate of the relative contribution of suspended and settling PON to the organic nitrogen in bottom sediments, and may provide a way of assessing the importance of these processes in the vertical transport of particulate material.

Table 1 $\delta^{15}N$ and PON at the sea surface

Station	Lat	Long	PON (μg atom) N l⁻¹	$\delta^{15}N$ (‰)	$\Delta\delta^{15}N$ (‰)
5	10°59.3′ S	110°59.4′ E	0.57	6.0	1.6
			0.53	7.6	
6	13°57.1′ S	103°01.5′ E	0.34	6.8	2.2
			0.38	9.0	
7	17°01.5′ S	95°00.4′ E	0.31	7.3	0.7
			0.32	8.0	
8	20°01.5′ S	86°58.6′ E	0.41	8.3	0.3
			0.63	8.6	
10	10°00.4′ S	86°58.9′ E	0.40	5.1	1.0
			0.59	6.1	
12	0°03.5′ N	86°56.1′ E	0.41	8.5	
13	5°02.2′ N	86°58.1′ E	0.44	3.2	0.9
			0.50	4.1	
15	14°03.5′ N	86°58.1′ E	0.51	7.6	2.5
			0.57	10.1	
17	5°02.8′ N	107°29.0′ E	0.74	3.8	2.3
			0.81	6.1	
18	8°59.7′ N	110°28.5′ E	0.98	6.0	1.3
			0.89	7.3	
19	12°58.8′ N	113°29.1′ E	0.62	5.5	2.5
				8.0	
20	17°03.0′ N	118°12.0′ E	0.60	5.2	0.3
			0.66	5.5	
21	20°03.2′ N	120°30.4′ E	1.37	2.1	1.4
			1.10	3.5	
	Mean				1.4±0.8

^{15}N abundance is presented in terms of $\delta^{15}N$ as defined by the equation:

$$\delta^{15}N = \frac{(^{15}N/^{14}N)\ \text{sample} - (^{15}N/^{14}N)\ \text{standard}}{(^{15}N/^{14}N)\ \text{standard}} \times 10^3$$

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Pyroclastic sulphur eruption at Poás volcano, Costa Rica

P. W. Francis, R. S. Thorpe & G. C. Brown

Department of Earth Sciences, The Open University,
Milton Keynes MK7 6AA, Bucks, UK

J. Glasscock

48 Filey Street, Sheffield 10, Yorkshire, UK

The recent Voyager missions to Jupiter have highlighted the role of sulphur in volcanic processes on Io[1-7]. Although fumarolic sulphur and SO_2 gas are almost universal in terrestrial active volcanoes, and rare instances of sulphur lava flows have been reported[8,9], sulphur in a pyroclastic form has only been described from Poás volcano, Costa Rica[10]. Here we amplify the original descriptions by Bennett and Raccichini[10] and describe a recent eruption of pyroclastic sulphur scoria and ejected blocks that are characterised by miniature sulphur stalactites and stalagmites.

Poás volcano is a small composite volcano (height 2,700 m, basal diameter 20 km) with a deep central crater 1.5 km wide and 300 m deep, in the floor of which is a smaller crater 400 m in diameter containing a hot (~50 °C) crater lake. Intermittent eruptions take place from one or more sites within the crater lake. These are generally small geyser-like plumes of steam and mud, but are occasionally much more vigorous, ejecting large, altered blocks, and more rarely, fragments of juvenile material. Small eruptions were taking place at intervals of roughly 20 min through much of 1978, but seem to have ceased since September 1978.

Bennett and Raccichini[10] noted the presence of greenish sulphur particles up to 4.5 cm in diameter around the crater in 1977. The particles resemble basaltic scoria in size and shape, and their nature and distribution indicated that they have been erupted as a shower of molten particles. Bennett and Raccichini attributed the 'sulphur scoria' to a 'lake' of molten sulphur located beneath the water in the floor of the crater, and estimated that 'several hundred tonnes' of sulphur were lying on the surface around the crater.

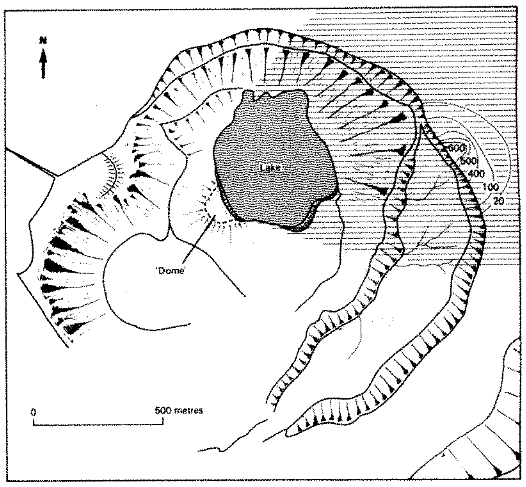

Fig. 1 Map of summit area of Poás volcano. Horizontal lines, area covered by ejected blocks. Numbered contours, surface concentration of sulphur particles in g m^{-2}.

We have carried out observations at Poás during January 1978 and February–March 1979. On the former occasion, frequent small eruptions and one large eruption took place; on the latter occasion, no eruptions were observed. In 1978 we were able to confirm Bennett and Raccichini's descriptions of scoriaceous sulphur particles dispersed widely, but sparsely, around and within the crater. At that time some of the sulphur was observed lying at, or near, the surface of an andesitic ash erupted in 1953 (ref. 11), and it therefore post-dates that eruption. Much the greatest proportion of the scoriaceous sulphur, however, had been washed downslope and had accumulated locally as deposits many centimetres thick. These deposits were striking on account of their greenish colour, and the shapes of the particles within them, some of which resembled Pele's tears which are characteristic of fluid basaltic eruptions[12].

In September 1978 a large eruption took place. Although poorly observed and recorded, due to persistent cloud covering the volcano, rangers of the Costa Rica National Park Service reported hearing loud explosions and seeing small, but widespread, blue flames around the crater rim after the eruption, and that the level of water in the lake had decreased sufficiently for

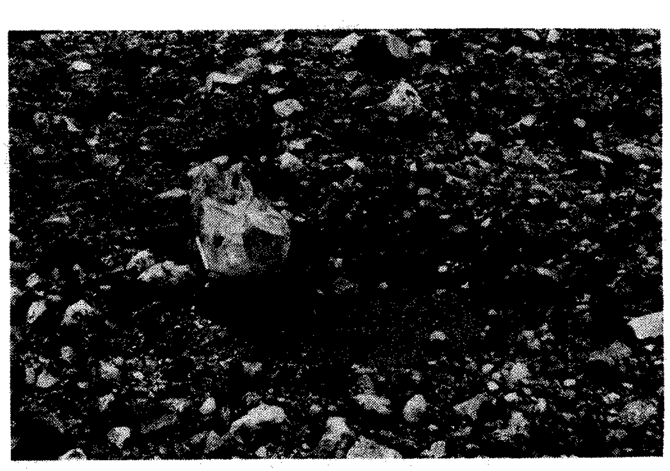

Fig. 2 Typical small ejected block and impact crater or eruption of September 1978. Note asymmetric position of block on crater rim; direction of travel was from right to left.

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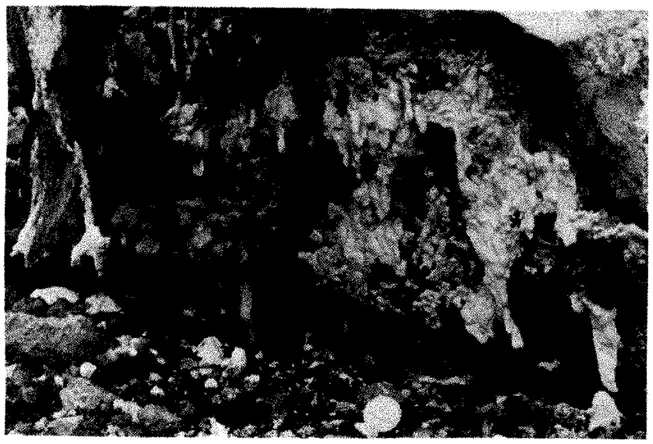

Fig. 3 Sulphur encrusted face of an ejected block, showing stalactites of sulphur. Stalactite and stalagmite nearly connect at right. The coin is 2.1 cm in diameter.

the bottom to be exposed in places. Our visit in February 1979 confirmed that at least one powerful explosive event had taken place, as the eastern flanks of the volcano were littered with fresh impact craters and large ejected blocks (Fig. 1). New fragments of green scoriaceous sulphur were abundant on the eastern rim of the crater, locally sufficient to cover the surface entirely. The distribution of the sulphur was studied by weighting the amounts found within individual 1-m square grids, and constructing a contoured map (Fig. 1). The greatest concentration of sulphur is on the east rim of the crater, whereas most of the older ashes have accumulated on the north and northwestern flanks, in response to the prevailing winds. This indicates that the sulphur particles were deposited by a directed blast, rather than falling from a wind-blown eruption cloud. A total of ~ 4 tonnes of pyroclastic sulphur particles lies on the surface examined, which suggests a total for the eruption of some 10–15 tonnes, allowing for sulphur which fell within the crater itself. Grain size analysis of the sulphur shows that, in size distribution and sorting characteristics, it resembles the basaltic ash of 1953 (unpublished data).

The asymmetrical distribution of the impact craters and ejected blocks from the September 1978 eruption also indicates a directed blast (Fig. 1). Blocks 0.3 m in diameter were found occupying craters 1.5 m in diameter several hundred metres from the crater rim. In most cases, the trajectories followed by the blocks were nearly tangential to the gently sloping flanks of the volcano and many blocks were therefore found lying at one side of their craters, or had bounded out of their initial craters and travelled onwards (Fig. 2). Measurement of the final resting places of the blocks relative to their impact craters enabled the azimuths of their trajectories to be estimated and the approximate site of initial ejection to be located. This corresponds closely with a breached dome on the crater floor, which is also the site of continuous intense fumarolic activity, and is heavily encrusted with fumarolic sulphur deposits.

Examination of the blocks revealed three distinct types to be present: (1) poorly consolidated lumps of grey clay and mud which had clearly been derived from the floor of the crater lake; (2) blocks of fresh, dark basaltic material which may represent the juvenile component involved in the eruption; and (3) heavily altered, sulphur encrusted blocks probably derived from the site of the active fumaroles. The sulphur encrusted blocks are concentrated towards the centre of the bombed zone, which corresponds closely to the area of maximum pyroclastic sulphur deposition. The distribution of the pyroclastic material is consistent with a powerful explosion having taken place at the foot of the cliffs at the present fumarole site, so that most of the ejecta were blasted out towards the east. A similar explosion may have taken place in 1964, as Krushensky and Escalante[13] described sulphur encrusted blocks in the ejecta from that eruption.

The unique aspect of the blocks ejected in 1978 is illustrated in Fig. 3. After coming to rest, the molent sulphur on the exterior surfaces of the ejected blocks dribbled downwards, dripping off projecting points to form stalactites, and accumulating on the surface beneath as stalagmites a few centimetres high. The formation of these features after the blocks came to rest is indicated by their vertical orientations, and by the formation of stalagmites on the loose ash and gravel of the preexisting surface. In a few cases, blocks were found exhibiting stalactitic features which were not vertical. These may have been produced during heating of the blocks before the eruption.

In contrast to the green scoriaceous sulphur resting on the surrounding surfaces, the sulphur encrusting the ejected blocks is strikingly yellow in colour. The difference in colour may represent an original difference in temperature of formation, or rate of cooling. An impression of the physical state of the yellow sulphur during stalactite formation is afforded by the fact that each stalagmite is formed of 20–30 individual pancake-like blobs of sulphur piled on top of each other and fused together. Clearly, the sulphur drips were at or near the melting point of sulphur, but the liquid sulphur was also of low viscosity.

As Sagan emphasised[5], the viscosity of liquid sulphur at its melting point is ~ 0.1 P but increases by a factor of 10^3 to a value exceeding 10^2 P between 157 and 187 °C. Thus while the sulphur on the ejected blocks cannot have greatly exceeded the melting point (110–119 °C), the green scoriaceous sulphur may have achieved temperatures of up to 200 °C, because the morphology and grain size characteristics of the fragments (unpublished data) are strikingly similar to those of true basaltic scoria formed from liquids with viscosities between 10^3 and 10^4 P (for example, data summarised in ref. 14).

Although it is curious that they have not been described before, the small stalagmites and stalactites on the ejected blocks seem to have a relatively straightforward origin. During the week before the large eruption of January 1978, we observed a marked increase (~ 10 °C) in the temperature of the fumaroles in the dome on the crater floor. The highest temperatures recorded were well above the melting point of sulphur. Presumably, a similar temperature rise preceded the eruption of September 1978, and was sufficient to melt the sulphur contained in a large volume of rocks surrounding the fumaroles. The molten sulphur accumulated in joints and fractures in the rock until the explosive eruption took place, and broke up the original mass into a large number of smaller blocks, and hurled them across the crater. Liquid sulphur still adhering to the joint faces of the blocks was partially stripped off in flight, but sufficient still remained on impact to dribble to the ground, forming stalactites and stalagmites.

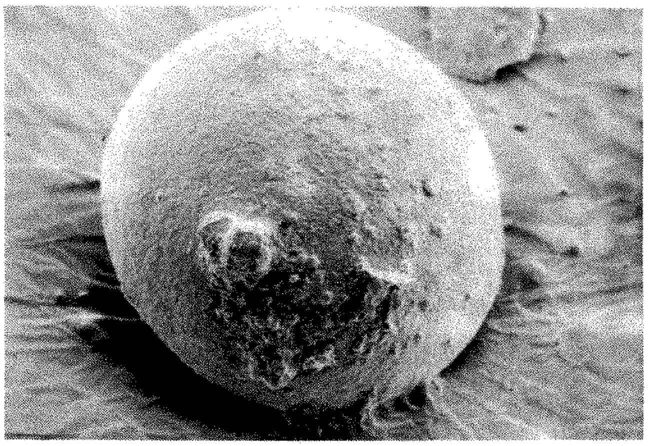

Fig. 4 Scanning electron micrograph of sulphur sphere from Poás lake mud. Sphere is 500 μm in diameter.

The scoriaceous sulphur is more difficult to account for. The 10–15 tonnes erupted in 1978 must have had a similar origin to that described by Bennett and Raccichini. They, however, reported the ejection of 'several hundred tonnes' of sulphur[10], which may be the products of several similar eruptions over a period of time.

We consider it unlikely that a 'lake' of liquid sulphur existed within the crater, as proposed by Bennett and Raccichini, but it seems probable that liquid sulphur congregated in fissures and pore spaces in the volcanic conduit beneath the site of the active fumaroles, and that this was sprayed into the air during the explosive eruption of September 1978.

The presence of water in the crater lake seems to have played an important part in this unusual eruption. Initially, it was responsible for sealing off the liquid sulphur from contact with the air, thus preventing its combustion or oxidation. Subsequently, interaction of the molten sulphur with lake water may have been responsible for the vigour of the eruption, and for the shaping of some of the sulphur particles. Evidence for this is the presence, within the lake muds and floating on the lake surface, of myriads of tiny spheres of sulphur (Fig. 4), which may have been produced when a spray of liquid sulphur was blasted into the lake water from beneath. We do not consider that temperatures as high as the boiling point of sulphur (444 °C) suggested by Bennett and Raccichini are necessary to explain the observed phenomena, particularly as the viscosity of liquid sulphur decreases between 200 and 444 °C (see ref. 5).

Although sulphur has an important tole in terrestrial volcanism, it is clearly dominant in ionian volcanic processes[1–7]. On Earth, sulphur and sulphur gases are an important component of explosive volcanic activity[15] and commonly form up to 30% of volcanic gases[16] which are removed from the atmosphere by surface processes. However, sulphur lavas and pyroclastic rocks as described here from Poás are relatively rare. On Io, sulphur dioxide is the dominant gas in the sustained volcanic plumes and sulphur may be a major component of the ionian crust[4,5]. Assuming that both have approximately chondritic Fe:S:Si ratios, the difference in surface sulphur concentration between Earth and Io presumably lies in their internal structures. Assuming the presence of a massive Fe–FeS core in the Earth[17] the magmatic source regions for terrestrial volcanic rocks must be relatively depleted in sulphur. On Io, by contrast, vigorous internal convection driven by tidal dissipation[18] might have been responsible for less efficient core formation and for less efficient partitioning of sulphur into its putative core.

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Rb–Sr age and source of the Bimodal Suite of the Ancient Gneiss Complex, Swaziland

J. M. Barton*, D. R. Hunter†, M. P. A. Jackson† & A. C. Wilson‡

*Bernard Price Institute of Geophysical Research, University of The Witwatersrand, 1 Jan Smuts Avenue, Johannesburg 2001, South Africa
† Department of Geology, University of Natal, Pietermaritzburg 3200, South Africa
‡ Institute of Geological Sciences, Keyworth, Nottingham NG12 5GQ, UK

The Ancient Gneiss Complex (AGC) in Swaziland[1–5] is a complexly deformed suite of gneisses that may be divided into three major units[5]: (1) the Biomodal Suite (BMS) of interlayered siliceous low-K leucocratic orthogneisses and amphibolites; (2) homogeneous tonalitic gneiss (the ~3,320 Myr old Tsawela gneiss)[6,7]; and (3) supracrustal rocks constituting two main lithologic sequences—the Mkhondo Valley[1,5] and Dwalile (ref. 8 and M.P.A.J. and A.C.W. unpublished data) metamorphic suites. The Tsawela gneiss is intrusive into both the BMS and the Dwalile metamorphic suite[8]. Relating the BMS to the greenstone sequence of the Barberton Mountain Land (the Onverwacht Group of the Swaziland Supergroup) has caused much controversy. It has been thought[1] that the BMS predates the Onverwacht Group and may represent a basement to the latter. Another view[9,10] is that all of the leucocratic gneisses of the AGC are younger than the Onverwacht Group and were derived from an unrelated mantle source. The amphibolite and metasedimentary rocks in the AGC are xenoliths of the Swaziland Supergroup. This controversy has remained unsolved due to the lack both of unambiguous contact relationships between the AGC and the Swaziland Supergroup, and of reliable radiometric data on the emplacement age of either unit. At the only known locality where these two units are juxtaposed, the contact is tectonically deformed. Early radiometric age data[6,11–20] have given results that are either too imprecise or reflect subsequent metamorphism[7]. Recently, however, a precise age of 3,510 ± 60 Myr (2σ) has been obtained by the Sm–Nd technique for extrusion of the volcanic rocks of the Komati Formation of the lower Onverwacht Group[21]. The results are reported here of Rb–Sr whole rock and biotite–whole rock isotopic analyses of samples of the leucocratic orthogneisses of the BMS. Results are also presented of initial ^{87}Sr/^{86}Sr ratio studies of samples of amphibolite from the Komati Formation. The relationship of the AGC to the Swaziland Supergroup is re-examined. All Rb–Sr ages mentioned are calculated using 1.42×10^{-11} yr^{-1} as the decay constant of ^{87}Rb.

The BMS has been intruded by the ~3,320 Myr old Tsawela gneiss[6,7], the ~3,030 Myr old Lochiel (Homogeneous Hood) granite (refs 15, 22 and J.M.B., unpublished data) and mafic dykes. Faulting and mylonisation in some areas have intensely deformed the BMS gneisses and fresh exposures of the BMS commonly look like smooth pavements. It is, therefore, difficult to obtain unaltered samples of the BMS in the type locality near Mankayane (Fig. 1). However, a quarry has recently been opened near the Fairview Dam in north-central Swaziland (Fig. 1) where the BMS is largely free from younger pegmatitic veins but is intruded by two generations of basic dykes. Vein-free samples of the leucocratic orthogneisses of the BMS were collected in this quarry at least 50 m from the dykes.

The analytical techniques used are described elsewhere[23,24]: the results are summarised in Table 1. Regressing the results of whole rock analyses of the leucocratic orthogneisses of the BMS[25] yields an isochron $(\mathrm{SUMS}/(n-2)=\mathrm{MSWD}=0.31<$

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Table 1 Analytical data

Sample	Rock unit	Rb (p.p.m.)	Sr (p.p.m.)	^{87}Rb/^{86}Sr*	^{87}Sr/^{86}Sr†	^{87}Sr/^{86}Sr‡
B-79-8A	BMS of AGC	77.3	256.	0.8596	0.7441	
B-79-8B	BMS of AGC	86.0	262.	0.9357	0.7483	
B-79-8B (bio§)	BMS of AGC	642.0	32.5	72.73	3.755	
B-79-8C	BMS of AGC	72.7	274.	0.7539	0.7389	
B-79-8D	BMS of AGC	97.9	222.	1.2592	0.7653	
B-79-8E	BMS of AGC	88.6	275.	0.9185	0.7475	
B-79-8F	BMS of AGC	97.3	365.	0.7583	0.7390	
B-79-8G	BMS of AGC	86.4	273.	0.9021	0.7466	
B-79-8H	BMS of AGC	71.3	272.	0.7468	0.7388	
B-79-8I	BMS of AGC	71.9	292.	0.7003	0.7363	
B-79-8J	BMS of AGC	86.7	267.	0.9274	0.7479	
B-76-40	Komati Fm.	1.167	26.3	0.1259	0.7064	0.7000
B-76-41	Komati Fm.	1.751	33.7	0.1473	0.7077	0.7002
B-78-19A	Komati Fm.	1.116	31.3	0.1011	0.7052	0.7000
B-78-19B	Komati Fm.	1.192	9.44	0.3589	0.7186	0.7003
B-78-19C	Komati Fm.	0.758	5.87	0.3674	0.7185	0.6997
B-78-19D	Komati Fm.	1.877	159.5	0.0344	0.7014	0.6996
AB9[11]	Komati Fm.			0.0311	0.7016	0.7000
AB9[1A]	Komati Fm.	0.272	40.9	0.0192	0.7014	0.7004
V36A[1]	Komati Fm.			0.0505	0.7023	0.6997
V36A[1A]	Komati Fm.	0.334	37.9	0.0255	0.7019	0.7006
AU20-37[1A]	Komati Fm.	0.882	20.0	0.127	0.7064	0.6999
VU32A[1A]	Komati Fm.	0.690	9.06	0.220	0.7115	0.7003

* Uncertainty = 1.4% (2σ)
† Uncertainty = 0.02% (2σ)
‡ Calculated for 3,510 Myr.
§ Biotite. The biotite–whole rock age was calculated from sample B-79-8B.
[11] Ref. 18. [1A] Ref. 19.

2.5) (ref. 26) of 3,555 ± 111 Myr (2σ) with an initial ^{87}Sr/^{86}Sr ratio of 0.6999 ± 0.0016 (2σ) (Fig. 2) (MSWD, mean square of weighted deviates). A biotite–whole rock age of ~2,889 Myr was also obtained.

The very low initial ^{87}Sr/^{86}Sr ratio of the whole rock isochron suggests that the calculated age corresponds closely to the time of emplacement of these rocks. This age is indistinguishable from the ~3,510 Myr age[21] of emplacement of the volcanic rocks of the Komati Formation, implying that these two units are radiometrically coeval. The leucocratic orthogneisses of the BMS, at least where sampled, cannot be a much older basement to the Onverwacht Group of an age similar, for example, to the > ~3,790 Myr old Sand River Gneisses of the Limpopo Mobile Belt[27]. The biotite–whole rock age implies that the major deformations manifested in the rocks of the BMS occurred by ~2,890 Myr ago. In view of their closeness, the major deformations displayed in the rocks of the Swaziland Supergroup, broad open and recumbent isoclinal folding[28], had also probably taken place by this time.

Unaltered samples of volcanic rocks from the Swaziland Supergroup have not yet been collected and all of the samples analysed so far have been metamorphosed to at least greenschist facies. The lack of deformation fabrics in many of these samples suggests that their mineral composition is not due to regional metamorphism in the conventional sense, but more probably to deuteric alteration. In addition, because no Rb–Sr whole rock isochrons corresponding to an age of ~3,510 Myr have been generated from samples from the Komati Formation, no direct determination of the initial ^{87}Sr/^{86}Sr ratios of these rocks have been made. Part of the scatter in the analytical results may reflect open system behaviour. However, in view of the ~3,430 Myr mineral isochron age derived from a single sample of the Komati Formation[19], the data scatter more probably reflects a range in initial ^{87}Sr/^{86}Sr ratios established within these rocks before this time. Part of this range may be attributed to chemical changes during deuteric alteration but some may also reflect a genuine range in ^{87}Sr/^{86}Sr ratios in the magmas themselves. Deuteric alteration generally increases the ^{87}Sr/^{86}Sr ratio of the rocks. Therefore, the maximum initial ^{87}Sr/^{86}Sr ratio of each sample may be estimated by subracting the radiogenic ^{87}Sr component that would have been generated over 3,510 Myr to leave the present ^{87}Rb/^{86}Sr ratio from the measured ^{87}Sr/^{86}Sr ratio. Results from the extrusive rocks of the Komati Formation have been treated this way in Table 1. Isotopic data[19] from sills and other differentiated intrusive bodies in the Komati Formation have been omitted because of uncertainty in their age with respect to the Komati Formation and their obviously altered composition[19]. The data from the extrusive rocks yield an average maximum initial ^{87}Sr/^{86}Sr ratio of 0.7001 ± 0.0006 (2σ). It seems, therefore, that the Komati Formation is not only coeval with the leucocratic orthogneisses of the BMS, but that the initial ^{87}Sr/^{86}Sr ratios of these units are indistinguishable.

These data are consistent with the possibility that the leucocratic orthogneisses of the BMS and the extrusive rocks of the Komati Formation are genetically related. It has been suggested from REE analyses[5] that these orthogneisses were derived from partial melting of basalt or amphibolite[29], having similar chemical compositions to the extrusive rocks of the Komati Formation. It is possible, therefore, that the rocks of the Komati Formation represent the parent that, on partial melting, yielded the leucocratic component of some of the BMS. The amphibolite in the BMS then could be remnants of the Komati Formation.

However, recent findings in southeastern Swaziland oppose this age relationship. In the western part of the Mankayane type area (Fig. 1), the BMS is in conformable contact with strongly distorted and disrupted metasupracrustal rocks, lithologically akin to the Onverwacht Group, known as the Dwalile metamorphic suite (ref. 8 and M.P.A.J. and A.C.W., unpublished data). These rocks have not been dated but are older than the ~3,320 Myr old[6,7] Tsawela gneiss that intrudes them (ref. 8 and M.P.A.J. and A.C.W., unpublished data). The original relationship between the BMS and the Dwalile suite is obscured by repeated episodes of high strain. Nevertheless, the metasupracrustal rocks are commonly preserved in the cores of synforms and no field relationships nor structures indicating that the BMS is younger than the Dwalile suite have been observed. Furthermore, the lower amphibolite grade of the rocks at Dwalile suggests a higher crustal level of metamorphism than that of the rocks of the BMS that are metamorphosed at upper-amphibolite facies (ref. 2 and M.P.A.J. and A.C.W., unpublished data). Available structural and metamorphic

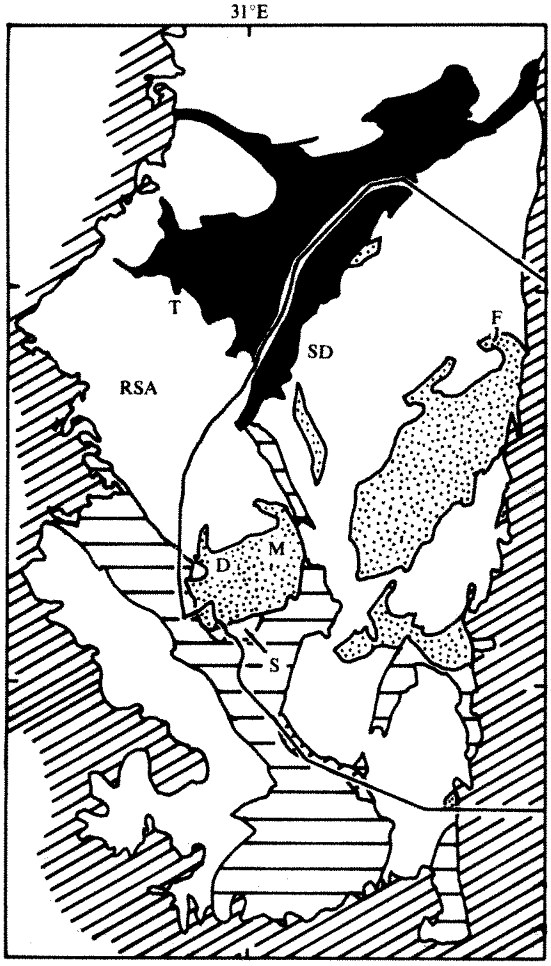

Fig. 1 A simplified geological map of the Barberton Mountain Land and the area to the south modified from Hunter[2]. ■, Swaziland Supergroup. ▒, Ancient Gneiss Complex. □, Younger granitoid intrusions. ▤, Pongola Group and the Ushushwana Complex. ▨, Transvaal and Karroo Supergroups. D, Dwailile; F, Fairview Dam; M, Mankayane; T, Theespruit pluton; S, Sicunusa pluton; SD, Swaziland; RSA, Republic of South Africa.

evidence therefore suggests that a basement–cover relationship exists between the BMS and the Dwalile suite. If this is true, it follows that the parent rocks of the BMS here could be older than the Komati Formation. The rocks classified in the BMS at the type locality may be different from those at the Fairview Quarry.

The initial $^{87}Sr/^{86}Sr$ ratio of the leucocratic orthogneisses of the BMS and those postulated for the Komati Formation are small and plot on the commonly proposed oceanic mantle Sr-isotopic growth curves[30,31]. These curves reflect oceanic mantle depleted in Rb with respect to Sr. Both the leucocratic orthogneisses and the Komati Formation may be derivatives of this type of mantle.

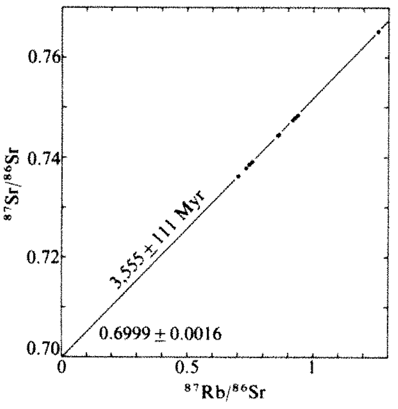

Fig. 2 Isochron diagram of the leucocratic orthogneiss of the BMS of the AGC.

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Mesozoic complementary crust in the North Atlantic

K. Rohr

Woods Hole Oceanographic Institution and Massachusetts Institute of Technology, Woods Hole, Massachusetts 02543

W. Twigt

Vening Meines Laboratory, University of Utrecht, The Netherlands

Detailed surveys of complementary oceanic crustal sections have been carried out over Mesozoic magnetic anomalies M4–M0 (~116–110 Myr BP) in the North Atlantic Ocean. We report here reconstructions of these crustal sections, one on the North American plate and one on the African plate, as they once existed together at the ridge crest using a statistical least squares technique, as well as by eye. The reconstructions allow a test of plate rigidity and consequently a refinement of the reconstruction of the whole North Atlantic in these times. Previous reconstructions had the uncertainty that the correct sections were being matched; the gentle curvature of the Mesozoic plate boundary provides little constraint along the strike of the boundary. The unique local pattern of the magnetic anomalies and their offsets in the surveyed areas allows an unambiguous match.

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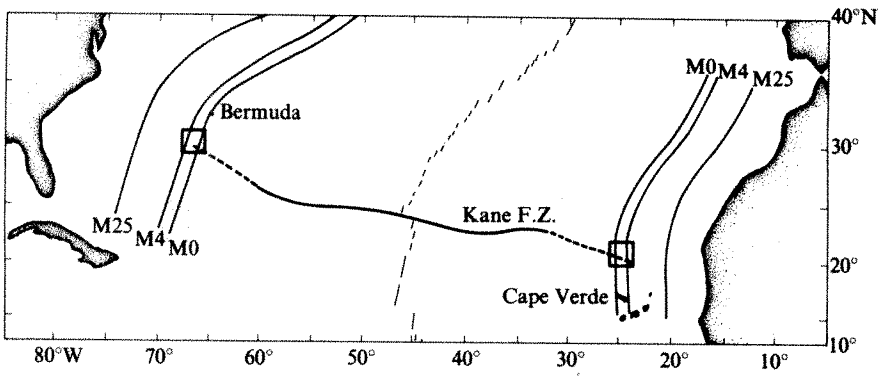

Fig. 1 Locations of two detailed survey sites.

On the North American plate a detailed geophysical survey of an approximate 1° square south of Bermuda was conducted in 1975 as part of the International Phase of Ocean Drilling[1] (Fig. 1). The additional magnetic data are from RV Atlantis II, RV Chain, USNS Keathley and a Project Magnet aeromagnetic survey[2]; fracture zone positions were picked from a Project Magnet magnetic anomaly contour map[3] on the basis of steep gradients, truncation of linear anomalies, and the presence of localised anomalies (Fig. 2*a*). The complementary crust on the African plate was surveyed by the Vening Meinesz Laboratory on board the MV Tyro in a 1° by 2° area north of the Cape Verde Islands in 1978 (Fig. 1). Additional magnetic and seismic data were obtained from the HMNS Snellius, RV Atlantic II, RV Vema, RV Conrad, USNS Gibbs, USNS Discoverer, and the USNS Kane. Fracture zone positions on the African plate were determined from seismic reflection data and picked as the deepest part of the trough (Fig. 2*b*).

The uniqueness of the magnetic pattern confirms that the two data sets are complementary. The survey on the North American plate showed that two left lateral fracture zones exist roughly 75 km apart and that the offsets across the fracture zones changed between M4 and M0 time. On the southern fracture zone the offset increased from ~15 to 35 km, and on the northern fracture zone decreased from ~40 to 30 km. These changes in fracture zone offset were accommodated by asymmetric spreading and rotation of the accretion axis[1]. An examination of the western Atlantic magnetic lineation map as compiled by Schouten and Klitgord[4] shows that this pattern of M4 and M0 lineations is unique. Finding the same pattern on the African plate, then, proves that the surveys are of complementary pieces of crust. On the African plate two left lateral fracture zones 75 km apart were surveyed; offsets for M4 and M0 were 15 and 35 km on the southern fracture zone and 40 and 30 km on the northern fracture zone (Fig. 2*b*).

On the African plate the location of the survey was predicted by rotating the North American data to Africa (Fig. 3*a*) by the M0 pole of Schouten and Klitgord (personal communication). This 'initial' Schouten and Klitgord pole is based solely on shipboard magnetic anomaly positions, the data set consists of ~60 anomaly identifications for each plate, spread over 23° in the central North Atlantic. In this reconstruction, the trends of the anomalies match quite well, but the predicted fracture zone positions fall roughly 20 miles south of the surveyed positions.

Poles of rotation which bring the estimated transform faults into better alignment were calculated for the two detailed data sets. Considering the density of data it seemed reasonable to use the least squares method of Hellinger[5] which gives a statistical best fit. Poles were also calculated from a visual fit to allow a comparison of methods.

The least squares method takes into account the estimated error for each data point; a computer program searches for the best fitting pole and angle of rotation within a specified area and range. The data are split into segments of spreading ridges and transform faults; data in a given segment from each plate are

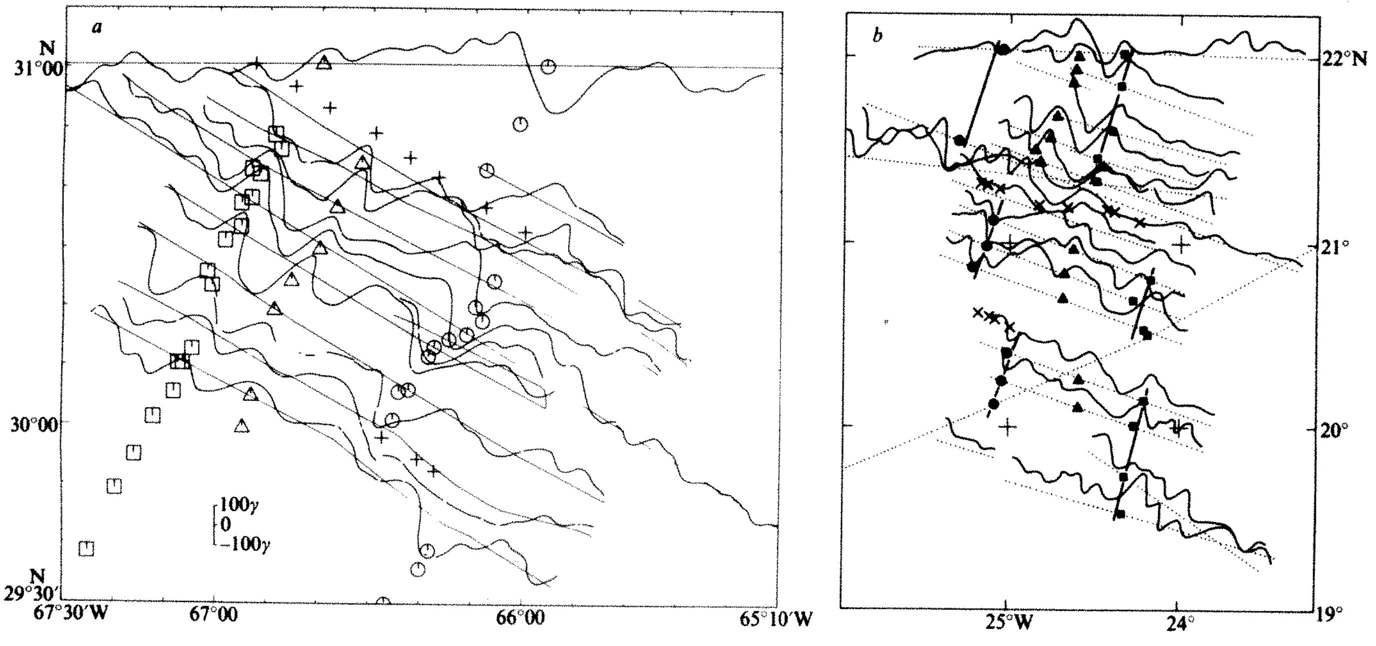

Fig. 2 Magnetic anomalies plotted along track with anomaly positions and fracture zone positions (+, ×) superimposed. *a*, North American plate; *b*, African plate. ●, M0; ▲, M2; ■, M4.

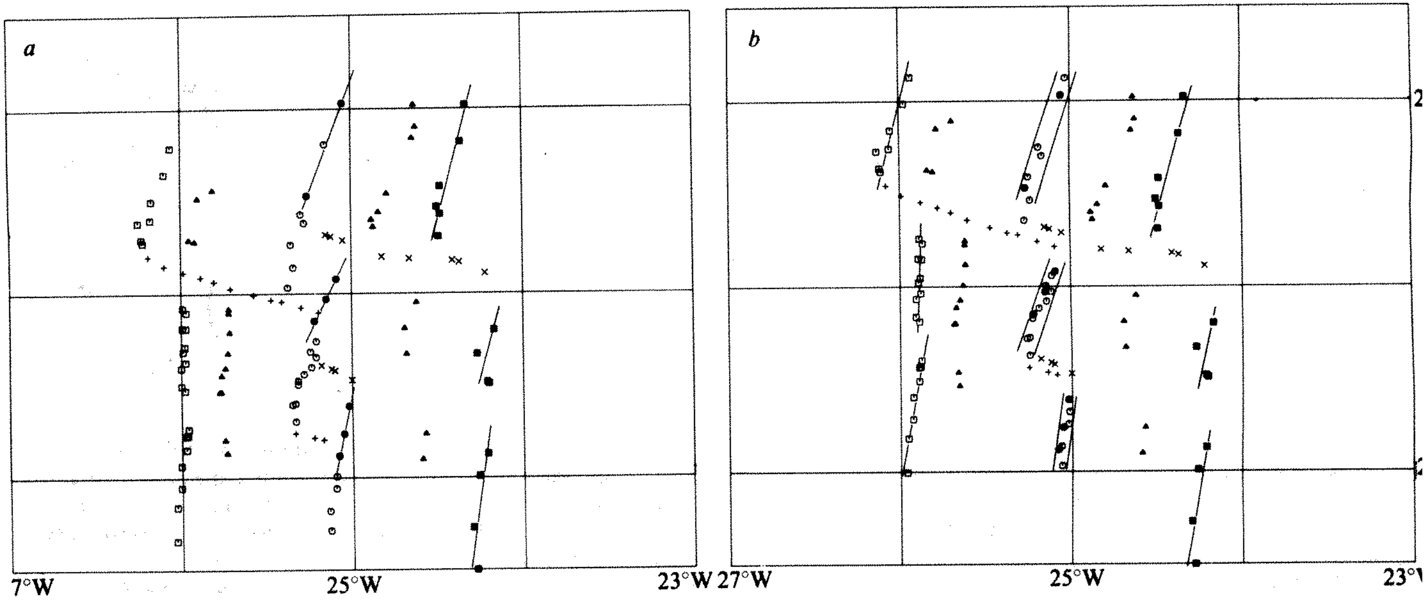

Fig. 3 *a*, Reconstruction of relative plate positions using the initial Schouten and Klitgord pole of rotation for M0. *b*, Reconstruction of relative plate positions using the least squares pole of rotation for M0. ○, M0; △, M2; □, M4.

matched. The data are rotated by a given pole and angle, and a great circle is computed for each segment of the plate boundary by a least squares technique. The closer the data fall on the great circle, the better the fit is considered to be.

Goodness of fit is measured by calculating the distance of a point from its great circle, squaring this quantity, and summing for all data points and all segments. This sum is called the fit measure and is computed for poles and angles within the specified ranges. The pole and angle with the smallest value of the fit measure is considered the best. Points known more accurately are preferentially weighted.

For these computations, magnetic anomalies were assigned an error in location of ±3 km, and transform faults ±10 km. The relative magnitudes of the errors are the important factor in the computations. The position of the transform fault active at a specific time within a fracture zone was considered to be less well constrained than the position of the magnetic anomaly isochrons. Uncertainties in ship navigation and variability in magnetic anomaly shape are such that the error in magnetic anomaly location is probably more realistically estimated as 6 km. Poles of rotation computed using an error of 10 km for the magnetic anomaly positions differed by a few tenths of a degree from those using an error of 3 km.

The data for magnetic anomaly M0 were divided into three ridge segments and two transform fault segments. The pole of rotation which gives the best fitting reconstruction of the M0 plate boundary is 66.78°N, 19.00°W with a rotation angle of 53.75° (Fig. 3*b*). We will call this the 'least squares pole'. The pole calculated from a visual reconstruction of M0 is 66.71°N, 19.25°W with a rotation angle of 53.85° (reconstruction not shown). (We call this the 'visual pole'.) In the visual fit fracture zone positions were made to be more coincident than on the rotation by the least squares pole. This condition is not necessarily valid as the North American transform fault positions were picked from magnetic anomalies and the African from seismic reflection profiles. The actual pole positions, however, are almost identical.

The M0 least squares pole of rotation and its contoured fit measure have been plotted (Fig. 4) along with the positions of the visual pole and the initial Schouten and Klitgord pole of rotation. The smallest fit measure for each pole on a 1° grid was plotted and contoured. A trough strikes roughly north-east–south-west containing poles with a low value of the fit measure, that is, poles that give 'good' or statistically acceptable fits. The 'best' pole lies within a local minimum.

Data for M4 were treated similarly (Table 1).

The poles calculated from the detailed data set for M0 and M4 by the least squares technique and the visual techniques are within $\frac{1}{2}$° or 1° of each other. The least squares techniques has the advantage of allowing a computation of the fit measure, a numerical description of how good each computed reconstruction is.

Both poles as calculated only from the detailed data sets were applied to the regional data and gave good fits for the whole ocean basin. The least squares pole gave a better fit than the visual pole. As a study of the fit of 2° of plate boundary has given a good description of the fit of 23° of plate boundary, the two plates must have behaved with a high degree of rigidity.

Schouten and Klitgord have since considered the detailed data and recomputed the M0 pole which also fits the regional

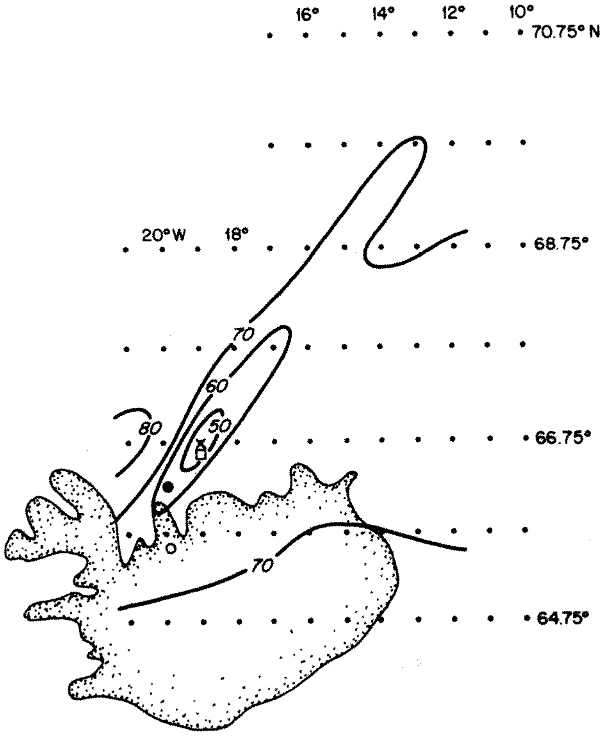

Fig. 4 Measure of fit for M0. The initial Schouten and Klitgord (○), the least squares (×), the visual (□), and the refined Schouten and Klitgord (●), pole of rotation positions have been plotted.

Table 1 Poles of rotation

	M0			M4		
	Lat	Long	Rotation angle	Lat	Long	Rotation angle
Initial Schouten and Klitgord	65.6	−19.9	54.71	65.3	−19.6	57.18
Least squares	66.75	−19.00	53.78	65.25	−21.00	57.55
Visual	66.71	−19.25	53.85	64.74	−21.55	58.11
Refined Schouten and Klitgord	66.3	−19.9	54.37	66.1	−19.0	56.40

magnetic data. This 'refined pole of rotation' lies 1° south-west of the least squares pole; it gives a statistically and visually satisfactory fit for the detailed data set. This suggests that poles of rotation based on widely scattered data can be improved by studies of small areas with a dense data coverage. This study further shows that in regions of sparse data, poles of rotation may be reliably calculated from detailed surveys of small areas of complementary crust.

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Is a black coat in the desert a means of saving metabolic energy?

Razi Dmi'el, Avi Prevulotzky & Amiram Shkolnik

Department of Zoology, The George S. Wise Faculty of Life Sciences, Tel Aviv University, Tel Aviv, Israel

Black goats of small body size (15–22 kg) dominate the herds of the Bedouins inhabiting the extreme deserts of the Middle East[1]; only a few white goats appear in such herds. Annual rainfall in these deserts varies from 15 to less than 150 mm; resources of food are sparse and watering points widely spaced. Summer is extremely hot and winter may be bitterly cold. Cloudy days are rare and solar radiation is immense all the year round[2]. Presumably, special adaptations would have been required in order to thrive in these harsh deserts. Can the black colour of the Bedouin goat be considered such an adaptation? Here, we have attempted to answer this question, and have found that by absorbing more of the Sun's energy on cold days, the temperature of the coat dramatically increased and the goat's oxygen consumption decreased significantly.

Short-wave radiation has been shown experimentally to penetrate animal coats by multiple diffuse reflection. For white coats, short-wave radiation may be scattered into the coat and absorbed below the surface, facilitating the inward flow of heat to the skin. For black coats, short-wave radiation is absorbed near or at the fur surface; little is reflected but a large amount is re-radiated as long-wave radiation before it reaches the skin[3,4].

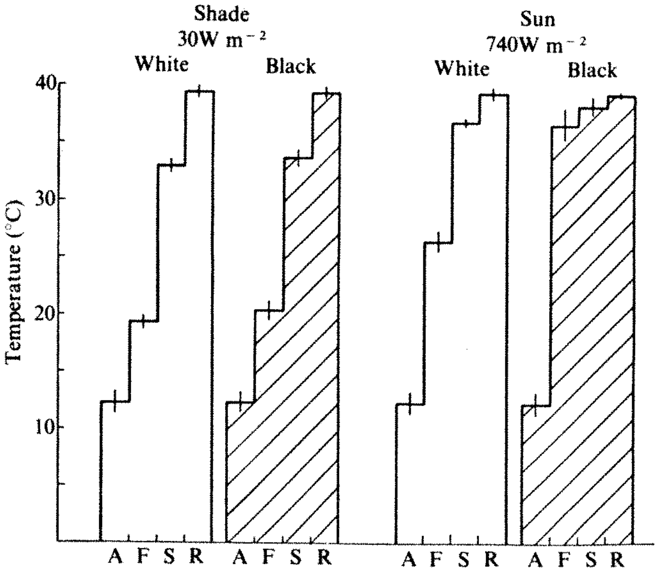

Fig. 1 Ambient and body temperature of two black and two white Bedouin goats, as measured during a clear winter day in the desert. Experimental time 0930 to 1430 h. Values are mean ± s.e. (vertical bars) of 35 measurements. A, Air; F, coat; S, skin; R, rectal.

This suggests that the black coat may thus reduce the heat gain and the need for evaporative cooling. However, recent studies have negated this possibility. It has been shown that the black goat absorbs about 80% of the solar radiation impinging on its fur and evaporates appreciable amounts of water, mainly through its skin[5–7]. In contrast, white goats absorb only 40% of the impinging radiation, and, consequently, evaporate much less water[6]. Black colour in the Bedouin goats is therefore not advantageous during the hot desert summer. The purpose of the present study was to determine whether their black coat enables the goats to improve their energy balance during the desert winter, when energy demands of the goat are high but food availability is at a minimum[8].

To assess the contribution of coat colour to the energy economy of the goats, we set out to answer two questions: (1) what is the effect of solar radiation on body temperatures and metabolism of Bedouin goats during a typical winter day in the desert, and (2) is this effect the same in black and white goats? Experiments were carried out during the winters (mid-December to mid-February) of 1976–8 in the mountainous region of the Sinai Desert. Two black, mature females (18.5 kg body weight) were purchased from local Bedouins. To ensure acclimatisation, they remained in their original herd for the entire year, except for the experimental period (12 measurement days altogether), when they were taken to the nearby study area. In addition, two local white goats were studied. They were also kept in the same herd as the black ones. Oxygen consumption, rectal, coat surface and skin temperatures of the goats were measured simultaneously, as the goats stood quietly either fully

Table 1 Oxygen consumption of black and white Bedouin goats during a clear winter day in the desert

	Oxygen consumption ($ml\ O_2$ per kg per h) Shade	Sun	Metabolic reduction (%)
Black goat	488 ±20	351 ±28	28.1
White goat	488 ±18	436 ±17	10.7

Values are mean ± s.e. of 35 measurements. Radiation in the shade 30 W m^{-2}, radiation in the Sun 740 W m^{-2}. Air temperature in both experimental conditions was 12.2 °C.

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Table 2 Changes in coat temperature and metabolism of black and white goats, as a result of transfer from shade to Sun

	Change in metabolism* (ml O_2 per kg per h)	Change in coat temperature† (°C)	Rate of metabolic change (ml O_2 per kg per h per °C)
Black goat	137	16.8	8.1
White goat	52	6.9	7.5

Air temperature in both experimental conditions was 12.2 °C.
* From Table 1.
† From Fig. 1.

exposed to the Sun or in the shade. The techniques and their accuracies were the same as those described in detail elsewhere[5,6], except that in the present study the animal and air temperatures were measured by fine (36-g) copper–constantan thermocouples. The measurements were made between 0930 and 1430 h, when air temperature (mean 12.2 °C, s.d. 3.5 °C) and solar radiation (mean 740 W m^{-2}, s.d. 95 W m^{-2}) were fairly stable. All the measurements were made over 30-min intervals; a period of 45–60 min was allowed to ensure thermal equilibrium before each experiment.

No difference in either body temperature or metabolic rate was found between black and white goats as long as they stood in the shade. However, when exposed to the Sun, the coat and skin temperatures of both black and white goats increased and their metabolic rate decreased. These changes were far greater in the black goats (Fig. 1, Table 1). As air temperature was the same in both Sun and shade, it is clear that the change in oxygen consumption depends on the change of the gradient between core body to coat surface temperature. The change in oxygen consumption per degree of temperature change is much the same in both goats (Table 2). This indicates that the differently coloured coats have similar insulative characteristics. Therefore, the larger metabolic reduction shown by the black goat is due to a greater change in its coat surface temperature, resulting from a higher absorption of the solar radiation.

To assess the role of black colour in a desert animal, one should take into consideration the entire climatic cycle to which the animal in question is exposed. We propose here that the critical season of the year for the Bedouin goat is the relatively short, cold desert winter and not the hot, dry summer. Presumably, water is not the crucial selection factor, because this breed is equipped with specialised physiological mechanisms which enable the goat to withstand prolonged periods of water deprivation[9,10]. On the other hand, these goats are not equipped with efficient mechanisms to prevent heat losses when exposed to cold. The loose and shaggy coat of the goat is a poor insulator. This is reflected in the rather high, lower critical temperature (26 °C) obtained for this breed[9]. Therefore, to keep its body temperature constant during cold weather, the goat must rely mostly on metabolic energy rather than on insulation. The reduction in metabolic rate, associated with absorption of more solar radiation by the black coat, can therefore be regarded as a mechanism which may be critical for the goat's survival during periods of food scarcity in winter. It is reasonable to assume, therefore, that selection pressure for survival of the Bedouin goat in the desert has centred on energy economy rather than water economy. The finding that black goats need less energy provides a logical explanation for the seeming paradox that the Bedouins have selected black animals for their milk and meat producers in hot deserts.

Black robes of goat's hair are commonly worn by Bedouins in the desert. A recent study carried out during the hot desert summer has demonstrated that the effect of a black robe on the heat balance of man is the same as the effect of a white one[11]. However, in the light of our measurements, it would be interesting to determine whether during winter in the desert, the black robe has a role similar to that we assign to the black fur of the goat.

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Role of the surface carbohydrates in sperm–egg interaction in *Ciona intestinalis*

Floriana Rosati & Rosaria De Santis

Stazione Zoologica, 80121 Napoli, Italy

In the ascidian *Ciona intestinalis*, species-specific recognition and binding between spermatozoa and eggs occurs at the outer surface of the vitelline coat (the chorion). Attachment of spermatozoa to the chorion occurs via very thin, Ruthenium red-positive fibrils connecting the plasma membrane of the tip of the spermatozoon with the fibrillar tufts of the outer surface of the chorion[1]. Experiments with fluorescein-conjugated lectins suggest that glucose or mannose and fucose residues are exposed at the surface both of the chorion and of the spermatozoon, while *N*-acetyl-glucosamine is only present at the surface of the chorion[2]. Work presently underway shows that the prevailing sugar components of the chorion are fucose, glucose and *N*-acetyl-galactosamine. However, this last sugar does not seem to be exposed at the surface of the chorion[2]. The role of the sugar residues exposed at the surfaces of the chorion and spermatozoon in gamete recognition and binding has been investigated to determine whether these sugars are functional components of the gamete receptors. It would be expected that binding of the spermatozoa to the chorion and fertilisation should be competitively inhibited in the presence of excess fucose, glucose and/or mannose, while *N*-acetyl-galactosamine and *N*-acetyl-glucosamine should not be effective. Our present results suggest that fucose residues play a key part in the binding reaction and in fertilisation.

For a quantitative estimate of the binding reaction we have used 'glycerol-treated' eggs (ref. 3). We have previously shown[1,3] that treatment of unfertilised eggs with glycerol does not impair the species-specific binding of the spermatozoa to the chorion. However, in these conditions the spermatozoa fail to undergo the acrosome reaction. This has allowed us to dissect out the events of sperm attachment from those of the acrosome reaction and sperm penetration through the chorion[1].

For a quantitative study of the effect of the various sugars on gamete interaction we have used the subtraction method of Vacquier and Payne[4]. The sperm concentration is linearly related to absorbance at 340 nm. The binding was routinely checked in the light microscope under dark field illumination[3]; this showed uniform distribution of spermatozoa attached to the eggs at all sperm concentrations used. The maximum number of spermatozoa attached to eggs occur between 3 and 6 min after 'insemination' (Fig. 1). The maximum percentage of bound spermatozoa is between 20 and 40% depending on the concentration of free spermatozoa. By varying the concentration of

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sperm available per egg, the maximum number of bound spermatozoa can be seen to be reached at a ratio of about 8×10^3 spermatozoa per egg (Fig. 2); in these conditions the estimated number of spermatozoa bound to each egg is about 2,500–3,000. This ratio was used in experiments of competitive inhibition with the sugars.

Preliminary experiments also showed that none of the sugars tested, even at the highest concentrations affect either sperm motility or oxygen uptake (Yasumasu, personal communication). However, the effect of these sugars is completely reversible. The effect of the different sugars on the binding reaction is shown in Fig. 3. Of the sugars tested, L-fucose is proved to be the only one which effectively competed with the binding of the spermatozoa to the chorion. The L-fucose concentration required for maximum inhibition is 50 mM; at 25 mM inhibition is approximately halved; at 10 mM this sugar is completely ineffective. These concentrations may, at a first glance, seem high. However one should consider that at the site of contact between spermatozoon and the receptors on the chorion the concentration of the sugar is likely to be very high. Similarly the interaction between lectins and their receptors is inhibited only at high sugar–lectin ratios.

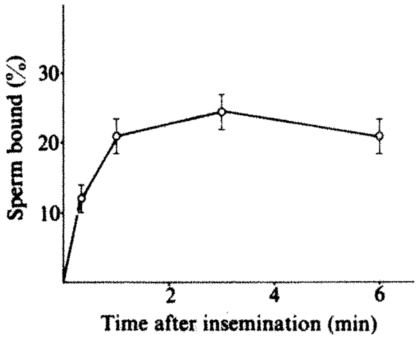

Fig. 1 Samples, each 1 ml, of a 5% suspension of glycerol-treated eggs (corresponding to 5×10^3 eggs) were 'inseminated' in small beakers with 1 ml of sperm suspension at a concentration of about 3.5×10^3 sperm available per egg. At the times indicated, 2 ml of 2% glutaraldehyde in sea water was added (which caused the eggs to sink rapidly to the bottom of the beaker). The absorbance of the supernatant (unbound spermatozoa) was then measured. The number of bound spermatozoa was calculated as the difference between the unbound and the total number of spermatozoa in the original suspension. The mean and standard deviations are calculated from four experiments.

A puzzling observation for which we have no explanation is that when fucose-treated eggs were checked under the microscope there was always about 10% of the eggs whose chorion was covered by attached spermatozoa (those which were not removed by repeated washing[3]), while the remainder did not have any attached spermatozoa at all. Even though the percentage of these eggs is small it is nevertheless bound to lower the value of the overall effect of fucose on sperm binding. When D-fucose, the uncommon isomer of L-fucose was tested under the same conditions, no effect whatsoever was observed. The results with glucose were variable; in some experiments the inhibition of binding ranged from 60 to 80% while in other experiments there was no effect. Figure 3 also shows that mannose, N-acetyl-glucosamine and N-acetyl-galactosamine have no inhibitory effect. The effect of galactose, although not present either in the chorion or in the sperm plasma membrane was also tested and proved to be completely ineffective.

We have also tested the effect of saccharides on fertilisation. In each experiment samples of 100 eggs from the same animal were suspended in the same concentrations of sugars used for inhibition of binding experiments. After 30 min of incubation the same amount of sperm (2–6×10^5 spermatozoa ml^{-1}) was added and 1 h later the eggs were checked for cleavage. The inhibitory effect of the L-fucose on the fertilisation response was proportional to the sugar concentration. In 15 experiments out of 18, 100% inhibition was observed at the highest concentration (50 mM) while at the lowest one (10 mM) there was no difference with respect to the controls. The inhibition ranged from 20 to 50% when the sugar concentration was 25 mM. The effect of glucose was again variable; in the majority of experiments from 40 to 100% of eggs were fertilised while in a few cases none were fertilised. The other sugars tested (mannose, galactose, N-acetylgalactosamine and N-acetylglucosamine) were totally ineffective. The effect of fucose is reversible; indeed binding and fertilisation occur normally when eggs incubated in fucose are then washed and inseminated. In another series of experiments, living eggs were inseminated in the presence of L-fucose; an aliquot was transferred to a dialysis tube and dialysed against sea water for 1 h. The eggs recovered from the dialysis tube proved to be fertilised while the undialysed controls did not cleave. The effect of fucosidase as well as of other glycosidases is now being tested.

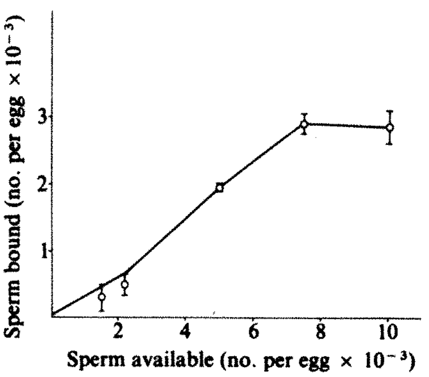

Fig. 2 Experimental conditions were as for Fig. 1. A constant number of eggs (5×10^3 ml^{-1}) was exposed to different concentrations of spermatozoa for 6 min, whereby the final sperm egg ratio (sperm available per egg) ranged 1.7–10×10^3. The mean and standard deviations are calculated from four experiments.

The results described here strongly suggest that L-fucose residues present on the plasma membrane of the spermatozoon and/or on the outer surface of the chorion are functionally involved in the recognition between spermatozoa and eggs. Interpretation of the molecular mechanisms involved must await further work. However in view of the presence of the same sugar residues both on the surface of the chorion and on the plasma membrane of the spermatozoon (as suggested by our

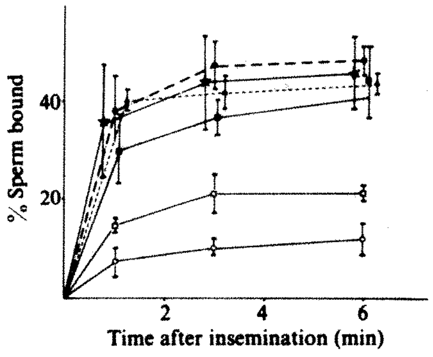

Fig. 3 Eggs (5×10^3 ml^{-1}) and spermatozoa (8–9×10^3 available per egg) were each suspended in sea water containing the sugar at a final concentration of 50 mM (unless otherwise indicated). The two suspensions were then mixed and the results checked at the time indicated in the abscissa. Fucose was additionally used at 25 mM concentration. In each experiment all sugars were tested and control experiments were performed using eggs and spermatozoa from the same stock. (All sugars tested were from Sigma.) The mean and standard deviations are calculated from six experiments. Control (●); fucose (○); fucose 25 mM (□); mannose (▲); N-acetyl-glucosamine (★); N-acetyl-galactosamine (■).

previous work[2]) a possible model might be the one proposed by Cook[5] for carbohydrate–carbohydrate interactions in all hydrated phase-containing carbohydrates and Ca^{2+}. The model is based on the observation that fucose and, in fact any uncharged sugar can form sugar–$CaBr_2$ complexes in which Ca^{2+} is bound to two symmetry-related sugar molecules and to three water molecules, thus resulting in the formation of hydrated fucose–Ca^{2+}–fucose bridges.

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Ca^{2+}-dependent hormonal stimulation of ciliary activity

Pedro Verdugo

Center for Bioengineering and Department of Biological Structure, University of Washington, Seattle, Washington 98195

It has been demonstrated that calcium modulates ciliary activity in protozoa[1] and also in epithelial cilia of the mussel gill[2]. Changes in the frequency of ciliary beat in the oviduct of the salamander have also been associated with movement of Ca^{2+} across the cell membrane[3]. However, the control of ciliary activity in mammals is poorly understood and the function of calcium in mammalian cilia and its role in coupling hormonal effects to changes in ciliary activity have been only preliminarily investigated[4]. The evidence presented here suggests that the stimulus–response coupling of the stimulating effect of prostaglandin E_2 (PGE_2) on ciliary activity of the rabbit oviduct is carried out by release of intracellular Ca^{2+}.

To avoid artefacts introduced by uncontrolled changes in the viscosity of epithelial secretions, experiments were conducted in cultures of ciliated cells from the rabbit fimbria. Tissue was grown according to the method of Rumery *et al.*[5] which yields cultures rich in ciliated cells and almost devoid of secretory cells. The animals were pretreated with a single intravenous (i.v.) dose of 100 μg of luteinising hormone administered 10 h before explanting the tissue. A total of 24 cultures of oviductal ciliated cells were grown in Rose chambers containing Eagle's medium (Steinberger modification) with 10% horse serum, *p*H 7.5, and incubated for 36–48 h at 37 °C.

Ciliary activity was monitored by laser light-scattering spectroscopy. A description of the application and validation of this method in comparison with the measurement of ciliary motion by high-speed cinematography has been published elsewhere[6].

The cultured cells were first equilibrated in Hanks' solution, *p*H 7.3 at 37 °C for 30 min. The Rose chambers were then positioned in a Reichert inverted microscope modified for laser scattering spectroscopy. The attenuated and collimated beam of a Spectra Physics 124A He–Ne laser was directed at the culture chamber illuminating an area of approximately 8,000 μm^2 containing about 10,000 cilia. The laser light scattered by the moving cilia was collected by the objective lens of the microscope at an angle of 35° from the plane of the chamber. The changes in intensity of the scattered light were detected as current fluctuations by a photomultiplier tube (RCA-C7164R). The spectrum of the photocurrent fluctuations which directly gives the statistical distribution of frequencies of ciliary beat (see Fig. 1 inset), was processed on-line by a fast Fourier transform digital spectrum analyser developed in our own laboratory.

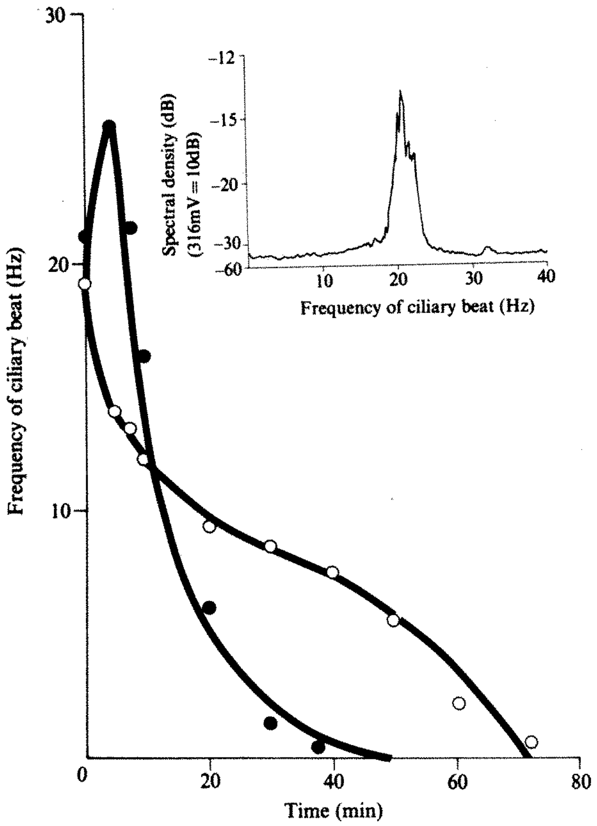

Fig. 1 Typical time course of the effect of decalcification on the frequency of beat of ciliated cells from the rabbit oviduct in tissue cultures. At time zero the cultures were perfused with Ca-free Hanks' solution containing 2 mM EGTA with (●) or without (○) PGE_2 (10^{-7} M). Note the initial increase followed by an exponential decrease in the frequency of ciliary beat produced by PGE_2. Inset shows the spectrum of ciliary beat obtained by laser scattering spectroscopy. Note the presence of a single dominant peak centred at a frequency of 21 Hz.

The cultures were first scanned to find clusters of ciliated cells demonstrating a stable and narrow peak of ciliary beat frequencies for at least 30 min. Following this control period, the Rose chamber was perfused with 10 ml of Ca-free Hanks' solution containing 2 mM EGTA. The frequency of ciliary beat was monitored for the next 60–80 min until ciliary arrest was observed (Fig. 1). The cultures were then re-equilibrated in Hanks' solution containing EGTA-buffered calcium concentrations of either 10^{-6}, 10^{-5}, or 10^{-3} M Ca^{2+} (6 cultures at each concentration). Aftef ~10 min the frequency of ciliary beat had increased to a new stable level which, in the case of the Hanks' solution containing 10^{-3} M Ca^{2+}, was approximately similar to the initial control. At this time the dose–effect relationship of PGE_2 (Upjohn) on the frequency of ciliary beat was investigated in each group of cultures by increasing the level of the prostaglandin in the Rose chambers while maintaining constant the correspondent calcium concentration (Fig. 2).

In a different set of experiments three cultures were decalcified in Ca-free Hanks' with 2 mM EGTA but in the presence of 10^{-8} M PGE_2 (Fig. 1). Finally in three other cultures PGE_2 was introduced into the Rose chamber after ciliary activity had been arrested by decalcification in Ca-free 2 mM EGTA Hanks' solution.

As observed in our preliminary investigations[7], the frequency of ciliary beat in mammalian ciliated cells can be reversibly modified by changing the extracellular Ca^{2+} concentration. However, the present experiments indicate that the stimulation of oviductal cilia by PGE_2 is fairly independent of calcium concentration in the medium. Note that in Fig. 2, especially at PGE_2 concentrations higher than 10^{-7} M, the rate of ciliary beat increases to a maximum and demonstrates no statistical difference over a rather wide range of Ca^{2+} concentrations.

The infusion of Ca-free Hanks' solution containing 2 mM EGTA in the Rose culture chamber produces a consistent

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biphasic time-dependent decrease of the frequency of ciliary beat (open circles in Fig. 1). However, when this same experiment is performed in the presence of 10^{-7} M PGE_2, the frequency of cilia beat shows a transient increase followed by an exponential decrease that results in the arrest of ciliary activity within approximately 40 min (solid circles in Fig. 1). This effect mimics that produced by the Ca ionophore, A23187, in cultures of ciliated cells of the rabbit trachea and oviduct[7].

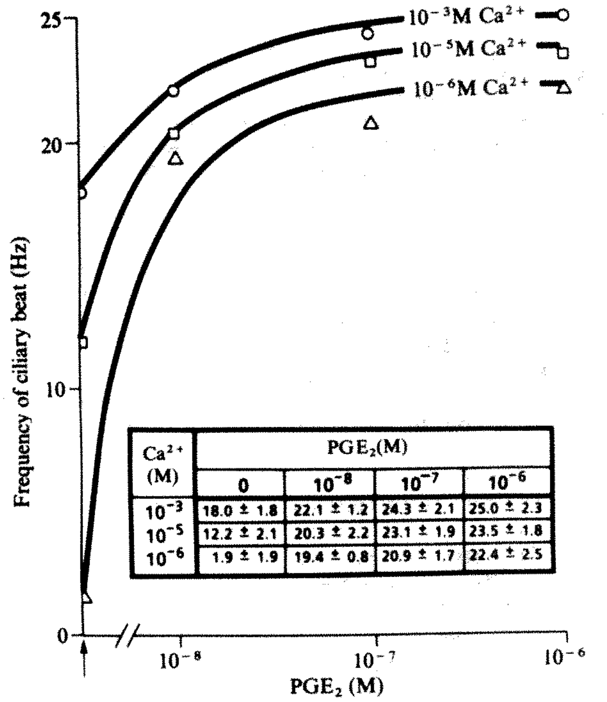
When PGE_2 at 10^{-7} M concentration is introduced into the culture chamber after ciliary activity has been arrested by 2 mM EGTA in Ca-free Hanks', a typical rapid increase followed by a rapid decrease in the frequency of ciliary beat is observed. Further increasing the PGE_2 concentration to 10^{-6} M produces transiently an additional smaller increase in frequency. However, this response could not be elicited again by 10^{-5} M PGE_2.

Calcium has been implicated in excitation–contraction coupling[8] and in coupling the avoiding response of ciliary reversal in protozoa[9] as well as coupling non-motile processes such as stimulus–release of neurotransmitter and endocrine hormones[10]. The present experiments suggest that calcium may also be involved in the stimulus–response coupling of hormonal stimulus to ciliary motions produced by PGE_2. It has been proposed that much of the pharmacology of prostaglandins can be understood on the basis of their facilitation of Ca^{2+} movement across biological membranes[11]. Our data appear to corroborate this idea, suggesting that the stimulation produced by PGE_2 must be associated with the release of intracellular calcium as: (1) the increase in the frequency of ciliary beat produced by PGE_2 is fairly independent of calcium levels in the medium (Fig. 2); (2) although the complex time decay in the frequency of ciliary beat produced by decalcification is not sufficient to support the idea of multi-compartmentalisation of calcium in ciliated cells, the transient increase, followed by an exponential decrease, in ciliary activity observed in the presence of PGE_2 (Fig. 1) is consistent with a prostaglandin-stimulated release of calcium from intracellular compartments; and (3) the transient activation produced by PGE_2 in ciliated cells maintained in Ca-free medium and arrested by calcium-depletion indicate that in these cells there must be a pool of intracellular calcium which can be released by PGE_2 and which may, transiently, stimulate ciliary activity before diffusing to the extracellular space.

The similarity of the effect of PGE_2 to that of the ionophore A23187 (Fig. 1) is not surprising as it has been suggested that prostaglandins may act as a 'calcium ionophore' in subcellular structures[12].

The data presented here are the first indication of calcium-dependent coupling of hormonal stimulus to ciliary activity. They suggest that PGE_2 stimulates ciliary motion by releasing intracellular bound or sequestered calcium, a mechanism that has also been suggested to explain the effect of prostaglandins in other tissues[12].

Prostaglandins have been found in the wall of the oviduct[13] where ciliary actions have been shown to be directly implicated in ovum transport[14]. The present work, therefore, may serve as a model to gain knowledge of the mechanism of action of hormonal influences on ciliary activity and also to understand further the role of prostaglandins in oviductal function.



Ca^{2+} (M)	PGE_2(M)			
	0	10^{-8}	10^{-7}	10^{-6}
10^{-3}	18.0 ± 1.8	22.1 ± 1.2	24.3 ± 2.1	25.0 ± 2.3
10^{-5}	12.2 ± 2.1	20.3 ± 2.2	23.1 ± 1.9	23.5 ± 1.8
10^{-6}	1.9 ± 1.9	19.4 ± 0.8	20.9 ± 1.7	22.4 ± 2.5

Fig. 2 Effect of various concentrations of PGE_2 on the frequency of ciliary beat in cultures of ciliated cells from the rabbit oviduct equilibrated in Hanks' solution containing various concentrations of calcium ions. Each point is the average ± s.d. of ~120 samples of frequency of ciliary beat.

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An ATP-dependent Ca^{2+}-pumping system in dog heart sarcolemma

Pico Caroni & Ernesto Carafoli

Laboratory of Biochemistry, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

Since the pioneering studies of Ringer of almost a century ago[1,2], it has been known that the contraction of heart cells requires the presence of calcium in the extracellular medium. Ca^{2+} penetrates into the sarcoplasm where it either directly activates troponin C (ref. 3), or indirectly conveys the contraction signal to the myofibrils, possibly by inducing Ca^{2+} release from intracellular stores (sarcoplasmic reticulum, SR)[4–6]. After contraction Ca^{2+} must obviously leave the heart cell again to prevent its progressive accumulation. As the electrochemical potential of Ca^{2+} across the sarcolemma would prevent its passive outflow, the efflux of Ca^{2+} is an energy requiring process. Most of the Ca^{2+} is apparently ejected from heart cells in exchange for Na^+ (refs 7, 8), on an electrophoretic antiporter which exchanges three Na^+ ions for one Ca^{2+} (ref. 9). However, there is some evidence[10–14] of a specific ATPase in cardiac sarcolemma, which could also have a role in the energy-dependent ejection of Ca^{2+}. One important difficulty in trying to establish the existence of a specific Ca^{2+}-ATPase in cardiac sarcolemma lies in the fact that most of the preparations used in such studies have been heavily contaminated by other subcellular fractions (mitochondria, SR) which also possess ATP-dependent Ca^{2+} transporting systems. In the present work we describe an ATP-dependent Ca^{2+} transport system which can be conclusively ascribed to heart sarcolemma. We show that a heart vesicular preparation particularly enriched in sarcolemma markers accumulates Ca^{2+} in the presence of ATP. That the process takes place in sarcolemmal vesicles is demonstrated by the fact that the accumulated Ca^{2+} is promptly and completely released in exchange for Na^+.

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Sarcolemmal vesicles from dog heart were prepared using a method slightly modified from that of Jones *et al.*[15]. The final pellet was washed twice and resuspended in 160 mM KCl, 20 mM HEPES *p*H 7.4 (K-medium), or, when required, in 160 mM NaCl, 20 mM HEPES *p*H 7.4 (Na-medium). Experiments on Na^+/Ca^{2+} exchange were carried out as described by Reeves and Sutko[8]. The $(Na^+ + K^+)$ATPase was measured after unmasking the total activity with Na-SDS[15]. The Ca^{2+}-ATPase was measured as described in ref. 15, in the presence of 20 μg oligomycin per mg protein. Each experiment described has been performed at least four times, with at least three different sarcolemmal preparations. The Na^+ content of the suspensions was determined by atomic absorption spectroscopy, and the protein content using a modification of the method of Lowry *et al.*[16].

The preparation used in the present work is very highly enriched in sarcolemmal vesicles, with specific activities of $(Na^+ + K^+)$ATPase varying between 80 and 105 μmol of phosphate liberated per mg of protein per h. The preparation accumulates Ca^{2+} at the expense of a transmembrane Na^+ gradient[8], 80% of the uptake (60 nmol per mg protein) taking place within the first 10 s. The sarcolemmal vesicles, however, also hydrolyse ATP in the presence of both Ca^{2+} and Mg^{2+}, with a specific activity of 8.5 μmol per mg protein per h. Concomitant with the hydrolysis of ATP, the vesicles accumulate Ca^{2+}, to a maximal level, in the experimental conditions used, of 46 nmol per mg of protein in 10 min (Fig. 1). The apparent K_m of this Ca^{2+} transport system lies between 0.2 and 0.6 μM.

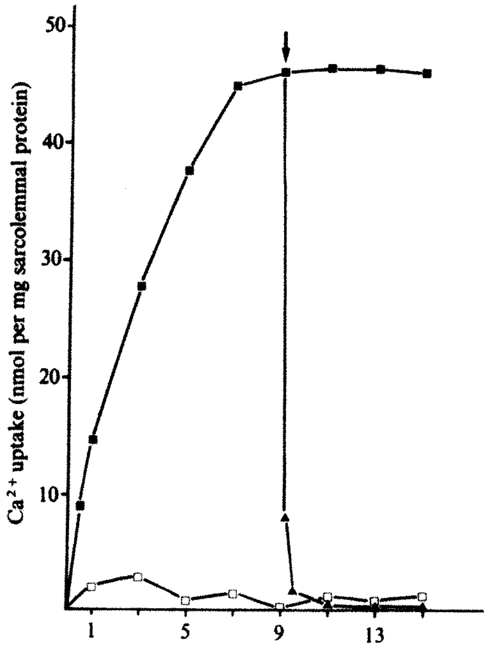

Fig. 1 ATP and Mg^{2+}-dependent Ca^{2+} uptake in dog heart sarcolemma. Sarcolemmal vesicles (20 μg of protein suspended in K-medium) were preincubated at 37 °C in 2 ml of a medium containing 160 mM KCl, 20 mM HEPES, 1 μg oligomycin, 5 mM $MgCl_2$, 1 mM ouabain *p*H 7.4. After 5 min, 150 nmol $^{45}CaCl_2$ were added: 2 min later, the first aliquot was withdrawn, and immediately after (time zero) 1 mM K_2ATP (final concentration) was added. Further aliquots (100 μl) were withdrawn at the times indicated, immediately filtered through Millipore filters (pore diameter 0.22 μm) and washed with 1 ml of ice-cold K-medium, containing 1 mM $LaCl_3$, to avoid Ca^{2+} movements during the 15-s filtration time. The values are corrected for the Ca^{2+} found associated to the sarcolemma before the addition of ATP (average, 28 nmol per mg of protein). The experiments were carried out at 37 °C. In none of the experiments was more than 20% of the total ATP added consumed. ■, No supplementary additions; ▲, at the arrow the ionophore A23187 (final concentration 5 μM) was added; □, no ATP or no Mg^{2+}.

Since two other membrane systems in heart—the mitochondria and SR—accumulate Ca^{2+} in the presence of ATP, appropriate controls were carried out. The possibility of the involvement of the mitochondrial system can be excluded, since oligomycin (20 μg per mg protein, an amount in great excess of that necessary to inhibit completely the ATP-dependent Ca^{2+} transport in mitochondria) and the uncoupler FCCP (1 μM) do not affect Ca^{2+} accumulation in sarcolemmal vesicles. Oxalate at 0.1 to 5 mM also had no effect, making the contribution of SR unlikely. Figure 1 also shows that Ca^{2+} is indeed accumulated into the inner vesicular space, and not superficially bound. The Ca^{2+}-specific ionophore A23187 discharges the accumulated Ca^{2+} completely and very rapidly. Also of interest is the observation that the accumulation of Ca^{2+} requires the presence of Mg^{2+} in the medium.

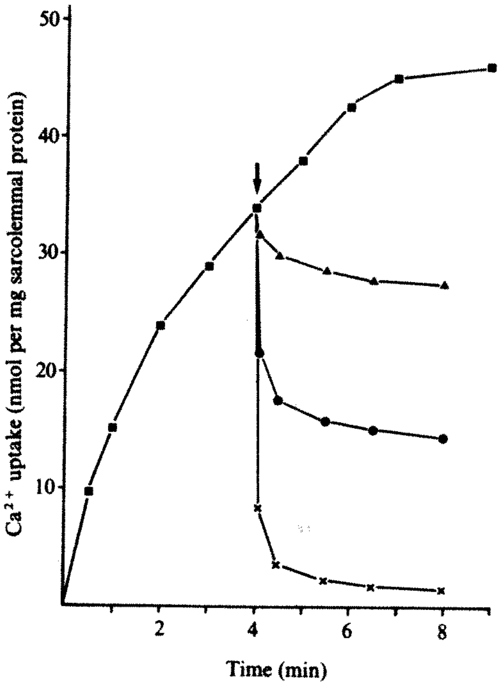

Fig. 2 Na^+-induced release of the Ca^{2+} accumulated by dog sarcolemma in the presence of ATP. Experimental conditions as in Fig. 1, except for the addition (at the arrow) of NaCl (or KCl) to give the final concentrations indicated below. ■, No additions, or 30 mM KCl, or 5 mM NaCl; ▲, 10 mM NaCl; ●, 20 mM NaCl; ×, 30 mM NaCl.

The sarcolemmal preparation used in the present experiments was derived from an oxalate-treated microsomal fraction. The possibility therefore existed that some oxalate could still be associated with SR vesicles contaminating the sarcolemmal preparation. Thus the ATP-dependent uptake of Ca^{2+} could be due, totally or in part, to the SR vesicles: the experiment shown in Fig. 2 rules out this possibility. The SR does not possess a Na^+/Ca^{2+} exchange system. However, the addition of Na^+ to the medium, after the vesicles have taken up about 35 nmol of Ca^{2+} per mg of protein, rapidly discharges the accumulated Ca^{2+}. The discharge is only partial at 10 mM added Na^+, but complete at 30 mM. Ionic strength and osmolarity effects are ruled out by the lack of effect of 30 mM KCl, demonstrating that the Ca^{2+} releasing effect of Na^+ is specific. It is clear, then, that the system under study is constituted by (sarcolemmal) vesicles into which an ATP and Mg^{2+}-dependent system pumps Ca^{2+}, and from which Na^+ specifically releases it.

One more possibility had to be ruled out before concluding that the sarcolemmal vesicles possess a Ca^{2+} transporting system that depends directly on the hydrolysis of ATP. Since it is to be expected that a considerable fraction of the vesicles used in this study have an inside/out polarity, the $(Na^+ + K^+)$ATPase could in principle have preloaded the sarcolemmal vesicles with Na$^+$, which could then be released in exchange for Ca^{2+} (ref. 9). Addition of large amounts of Na$^+$ could then release the accumulated Ca^{2+} again. Measurements of the Na$^+$ concentration in the outside medium, however, have indicated values below 20 μM, at which no (Na$^+$ + K$^+$)ATPase activity could be detected. In addition, the inclusion of ouabain in the reaction medium (followed by preincubation for 1 h at 15 °C to allow equilibration of the inhibitor across the vesicle membrane) had no effect on the observed movements of Ca^{2+} in the experimental conditions used. Finally, the inclusion in the medium of Na$^+$ concentrations increasing from 100 μM to 1 mM (lower than those necessary to observe any release of the accumulated Ca^{2+}, but already adequate to sustain a certain level of (Na$^+$ + K$^+$)ATPase activity[17]), failed to increase the uptake of Ca^{2+}. An increase in Ca^{2+} uptake, on the other hand, would have been expected if the accumulation of Ca^{2+} were due to the discharge of a Na$^+$ gradient previously established by the operation of the (Na$^+$ + K$^+$)ATPase. We can therefore conclude that the sarcolemma of heart cells possesses, in parallel with the Na$^+$/Ca^{2+} exchange system, an ATP-dependent system which specifically transports Ca^{2+}. This system seems to have a higher affinity for Ca^{2+} than does the Na$^+$/Ca^{2+} exchange system, but a lower transport rate (Fig. 2). These differences could be of importance in considering the respective roles of the two systems in heart physiology.

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Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle

G. D. S. Hirst & T. O. Neild

Neuropharmacology Group, Department of Physiology,
Monash University, Clayton, Victoria, Australia 3168

We have recorded the responses of arteriolar smooth muscle cells to iontophoretically applied noradrenaline. Records of both muscle movement and muscle membrane potential were made. We found that two distinct types of response could be detected, depending on the position of the noradrenaline micropipette. One type of response consisted of a localised constriction near the noradrenaline source: this effect could be abolished by the α-antagonist phentolamine and was not associated with a change in arteriolar membrane potential. The other type of response was a depolarisation similar to the excitatory junction potentials (e.j.ps) produced by sympathetic nerve stimulation. These observations suggest that there are two populations of receptors for noradrenaline on arterioles, and could explain the paradoxical failure of α-antagonists to block neuromuscular transmission at some autonomic end organs such as the vas deferens, arteries and arterioles.

Intracellular recordings were made from very short segments of arteriole (~0.25 electrical length constants; for details see refs 1, 2). Both the membrane potential and diameter of the arteriolar segment were monitored, the latter by use of an inverted compound microscope[1] and a video tape recorder. Muscle movements were measured after the end of the experiment by playing back the video signal frame by frame. The use of such short lengths ensured that any membrane potential change occurring in the preparation would be detected by the intracellular recording electrode. This point was checked by impaling the preparation with two independent microelectrodes, one at each end of the segment. Invariably if both recording electrodes detected an e.j.p. following nerve stimulation, passage of current through one electrode caused a change in membrane potential which was detected by the other electrode. With these very short preparations the electrotonic potentials could not be distinguished from those which would result from current injection into a simple resistance/capacitance circuit[2].

The normal response of such preparations to low frequency supramaximal nerve stimulation (0.4 Hz) was the initiation of e.j.ps with mean amplitudes in the range 1.8–12 mV. These potentials did not produce a detectable movement although occasionally an e.j.p. reached threshold and initiated a muscle action potential resulting in constriction of the whole segment. E.j.ps were not reduced in amplitude by either phentolamine mesylate, tolazoline hydrochloride, prasozin hydrochloride or labetalol hydrochloride (each at 1×10^{-6} g ml^{-1}) even at stimulation frequencies as low as 0.1 Hz.

Attempts were made to mimic nerve stimulation by iontophoretic application of noradrenaline from a second microelectrode (noradrenaline 0.5 M, electrode resistance 150–500 MΩ). The most common response to application of a brief pulse of noradrenaline (5–10 ms, 50 nA) was a localised constriction of the arteriole in the region near the iontophoretic pipette (Fig. 1). These responses were not associated with a change in membrane potential. There was no obvious relationship between the separation of the recording electrode and the iontophoretic electrode and the ability to produce localised constriction. In several experiments constriction of the arteriole occurred within a few micrometres of the recording electrode but even this was not accompanied by a change in membrane potential. The responses not associated with a change in membrane potential (Fig. 1) could be abolished by the addition of phentolamine mesylate (1×10^{-6} g ml^{-1}) to the bathing solution.

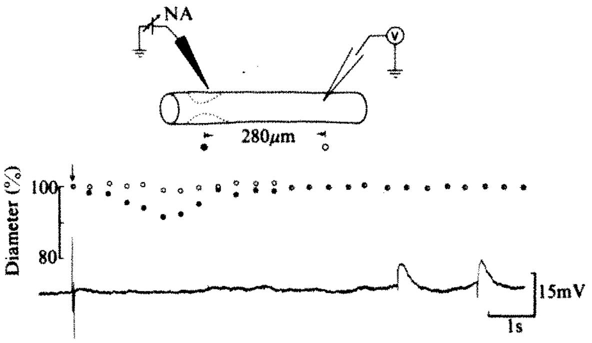

Fig. 1 Effect of iontophoretic application of noradrenaline (NA) on the diameter of an isolated arteriolar segment (upper trace, circles) and on its membrane potential (lower trace). The arteriole had a diameter of 63 μm and a length of 350 μm; after the application of noradrenaline (pulse duration, 10 ms; current, 50 nA at arrow) the arteriole constricted but the constriction was limited to the area near the iontophoretic pipette (●, see experimental sketch). No change in diameter was detected at the position of the recording electrode (○) which was placed some 280 μm further along the arteriolar segment. This response was not associated with a detectable change in membrane potential. At the end of the lower trace, two e.j.ps produced by perivascular nerve stimulation can be seen. These were not associated with a detectable change in arteriolar diameter.

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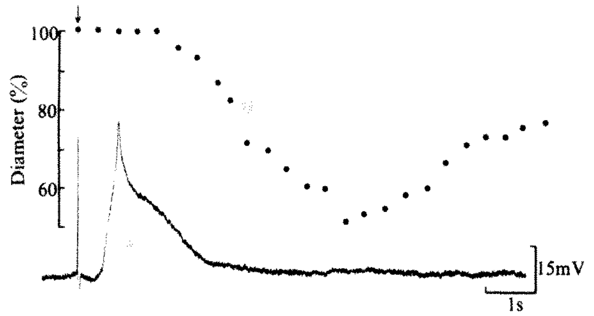

Fig. 2 Effect of iontophoretic application of noradrenaline at a 'reactive area'. A pulse of noradrenaline (duration 10 ms, current 50 nA) produced a depolarisation which initiated an action potential (lower trace), and a generalised constriction of the entire arteriolar segment (upper trace).

On some occasions areas on the arteriolar surface were found where iontophoretically applied noradrenaline produced a depolarisation (Fig. 2). The position of the iontophoretic electrode was critical; in any one experiment some 10–20 trials would be required before an area where noradrenaline caused a depolarisation was found. When this depolarisation initiated an action potential, the entire arteriolar segment constricted (Fig. 2). The delay between the start of the iontophoretic pulse and the onset of depolarisation was highly variable, ranging from <10 ms to 500 ms. Part of this delay is probably due to the difficulty of expelling catecholamines from high resistance microelectrodes because the delay could be shortened by using short high-frequency trains of iontophoretic pulses. Depolarising responses to iontophoretically applied noradrenaline could be detected in the presence of phentolamine (1×10^{-5} g ml^{-1}) but we have not yet been able to rule out the possibility that phentolamine had reduced the amplitude of the depolarisation. During a prolonged intense depolarising response (30–40 mV change in membrane potential) to noradrenaline produced by long iontophoretic pulses (100 ms) e.j.ps could not be detected. This suggests that neuronally released transmitter and applied noradrenaline may initiate conductance changes with similar reversal potentials.

There is an abundance of data to indicate that noradrenaline is synthesised, stored, and released by many sympathetic postganglionic nerve terminals[3]. However, in smooth muscle tissues which receive an excitatory noradrenergic innervation such as arteries, arterioles and vasa deferentia, it has been difficult to explain why responses to exogenously applied noradrenaline differ in some ways from those to nerve stimulation. For example, stimulation of sympathetic nerves innervating the vas deferens produces a two-component contraction, only one component of which is abolished by traditional α-receptor blocking drugs[4]. The contractile response produced by exogeneous noradrenaline can be readily abolished[5]. Noradrenaline causes a contracture of arteries in concentrations which do not produce a change in membrane potential[6]; but e.j.ps not associated with movement, are detected from arterioles and arteries in response to sympathetic nerve stimulation[7,8]. For reasons such as these the role of noradrenaline as the primary transmitter has been questioned[9,10].

We suggest that the difficulty in duplicating responses produced by neuronal stimuli by the application of exogenous noradrenaline arises because the smooth muscle cells have two distinct populations of excitatory receptors for noradrenaline. One group of the receptors which cause membrane depolarisation are the same receptors (junctional receptors) as those which are activated by neuronally released noradrenaline. The other (extrajunctional) receptors have the pharmacological properties of traditional α-receptors. When they are activated, they cause constriction without an associated membrane potential change. In arterioles either the neuronally released transmitter does not have access to extrajunctional receptors or if it does it reaches them in such a low concentration that no contractile responses can be detected (this may not be the case for more densely innervated tissues such as the vas deferens).

These observations are consistent with the view that noradrenaline is a primary transmitter in the sympathetic nervous system. However, responses to sympathetic nerve activity can only be mimicked if noradrenaline is applied to certain restricted areas; the receptors activated at these areas are different from conventional α-receptors which therefore seem to play no part in neuromuscular transmission.

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Proprioceptors with central cell bodies in insects

Peter Bräunig & Reinhold Hustert

Fachbereich Biologie, Universität Konstanz, Postfach 5560,
D-7750 Konstanz, FRG

A common feature of invertebrate sense organs is that the cell bodies of the sensory neurones are located in the periphery, close to the site of stimulus reception. For some phyla of invertebrates examples are known where sensory cells have their somata inside the central nervous system (CNS), but none are yet known for the insects. In this report we describe a proprioceptive organ in the locust thorax that consists of an elastic strand that is supplied with terminal branches of a small group of sensory neurones. The somata of these neurones are not located in the periphery, but inside the third thoracic ganglion. Our findings indicate that at least a small number of cell bodies in insect ganglia may belong to sensory neurones, rather than to motoneurones, interneurones or neurosecretory cells.

In locusts the hindlegs are involved in a number of behavioural tasks like stepping, stridulating, kicking, swimming, steering in flight, grooming and help in changing body position. These movements do not follow all-or-nothing programmes imposed by the CNS but are modified in amplitude and duration in response to sensory information especially that from the proprioceptors of the leg itself. Although the proximal joints between thorax, coxa and trochanter of the hindlegs have a major role in whole leg movements, more is known about the more distal joints between femur and tibia and tibia and tarsus[1,2]. We looked for additional internal proprioceptors in the proximal leg joints of the migratory locust (*Locusta migratoria*) by infusing cobaltous solutions distally[3,4] into nerves 2 and 3 of the metathoracic segment. One of the terminal peripheral branches of nerve 3 splits into a meshwork of arborisations on a connective tissue strand between the thorax and the trochantin (Fig. 1) without supplying any detectable somata. This is in contrast to nearby chordotonal organs (for example, cxtnCO in Fig 1). Backfills of these terminal arborisations on the strand show 6–8 neurone somata, 10–15 μm in diameter, in the anterior lateral region of the metathoracic ganglion (Fig. 2). These neurones show rather restricted fields of arborisation compared to locust motoneurones[5].

It seemed possible that this new structure was a degenerating larval muscle, but electron microscopic sections of the strand and terminal nerve arborisations did not show any similarity

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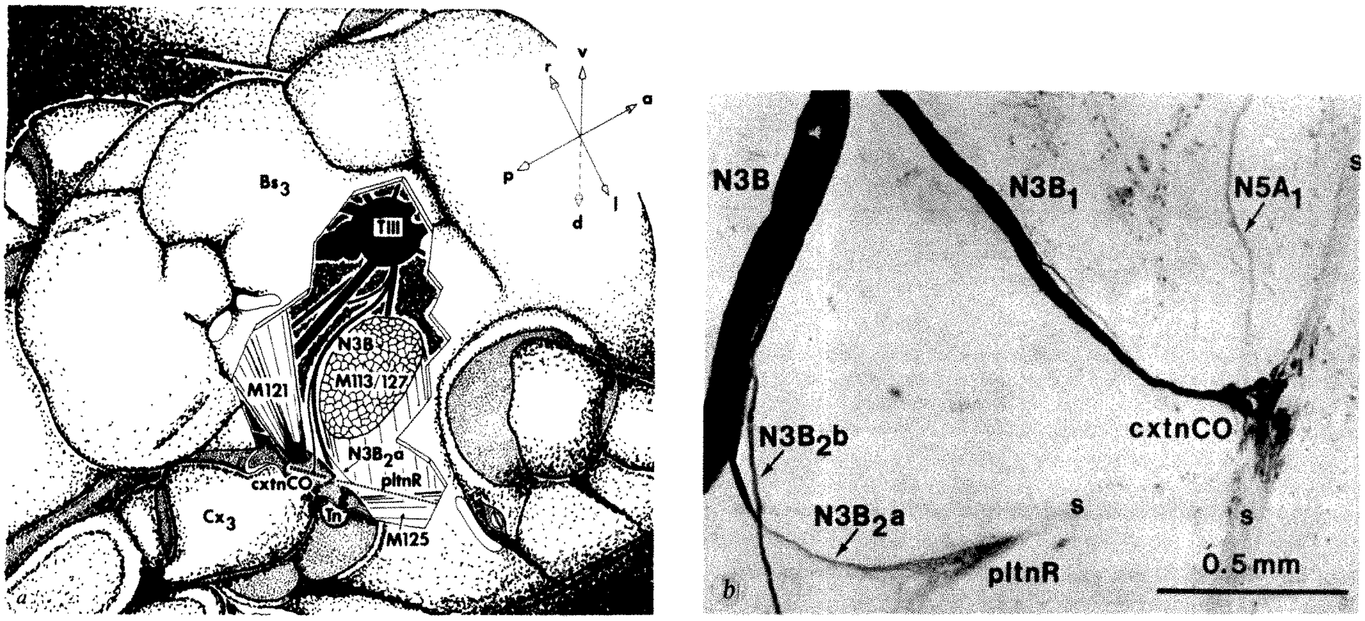

Fig. 1 Morphological features of the pleurotrochantinal receptor (pltnR) in the metathorax. *a*, Ventro-lateral view of the third thoracic segment (metathorax) of the migratory locust. A large window has been cut into the sternal cuticle (Bs_3) and the joint membrane (narrow stipling) of the hindleg coxa (Cx_3). Air sacs and trachea have been removed exposing some muscles (M121, M113/127, and M125) and the metathoracic ganglion (TIII) with all anterior nerve roots except for nerve 2. Two sense organs of the leg joint (cxtnCO, tnplR) are innervated by small branches which emerge from the middle tract of the third nerve root (N3B). a, Anterior; p, posterior; r, right; l, left; v, ventral; d, dorsal; Tn, trochantin. *b*, Peripheral cobalt stain showing branches of nerve 3B. Note the prominent cell bodies in the chordotonal organ (cxtnCO) in contrast to the receptor (pltnR); s, connective tissue strand. The relative position of the two sense organs does not correspond with the natural one in this microscopic wholemount.

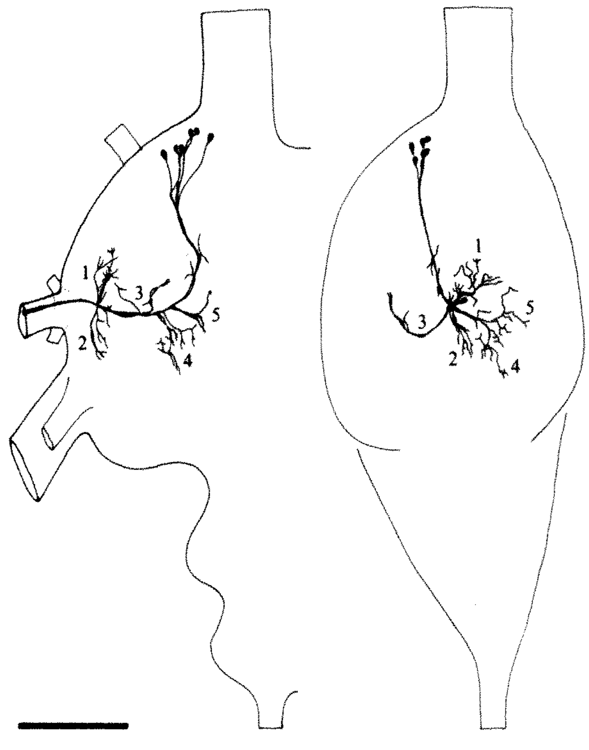

Fig. 2 Cobalt filled and silver intensified central projections in the metathoracic ganglion and somata of six neurones supplying the receptor. Camera lucida drawing of the hemiganglion, viewed dorsally and laterally. Same dendritic areas are indicated with corresponding numbers. Scale bar, 0.2 mm.

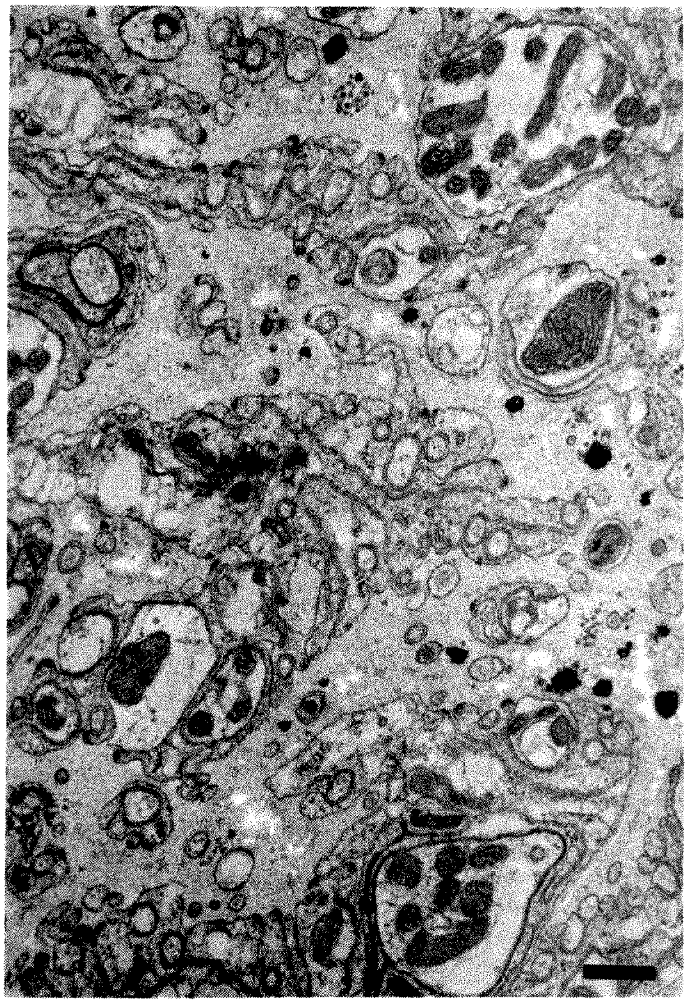

Fig. 3 (right) Electron microscopic cross-section of the receptor strand containing terminal branches of nerve $3B_2a$. Characteristic features: large (for example upper right) nerve profiles, usually surrounded by glia and containing many mitochondria, and small nerve profiles, wrapped in glia or embedded in extracellular material without glial sheath (middle). Scale bar, 0.5 μm.

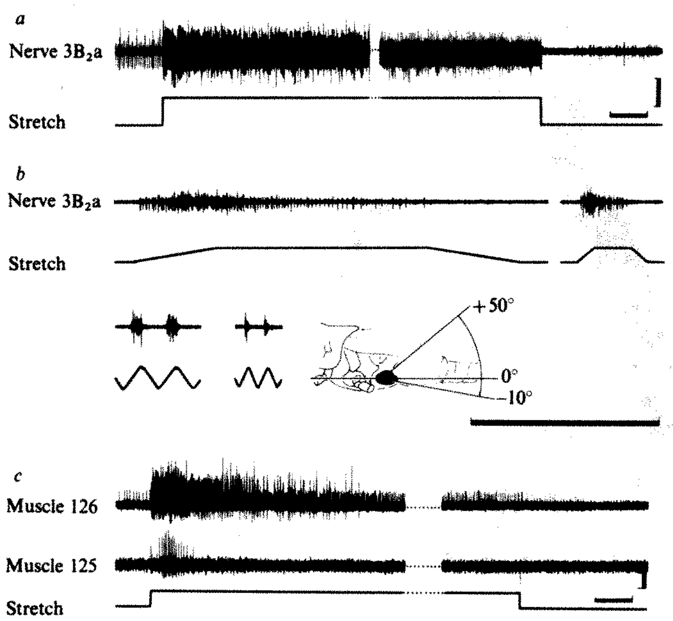

Fig. 4 Afferent and efferent responses to mechanical stimulation of the receptor. *a*, Phasico-tonic afferents (upper trace) after stretching (lower trace) the strand record from distal end of cut nerve $3B_2a$. Note the pronounced off-response. *b*, The effect (upper trace) of slow and fast trapezoid movements (lower traces) applied to the coxa from −10° to +50° as indicated. Range corresponds to normal range in behaviour. *c*, Phasico-tonic reflex response (upper trace) in the motonerve supplying muscles 125 and 126 and phasic response in a myogram of muscle 125 (middle trace) to stimulation of the receptor as in 3*a* (lower trace). Calibrations: horizontal, 1 s; vertical, 0.5 mm. Dotted lines are omitted parts on continuous recordings.

with muscle tissue (Fig. 3). The strand can be found in all larval instars and it persists in the adult without change.

Further evidence for a sensory function was obtained by recording from the distal part of the terminal nerve branch which supplies only the strand. Afferent volleys of spikes from the organ indicated the presence of several units responding in a phasico-tonic manner to trapezoid stretching of the strand (Fig. 4*a*). This identifies it as a stretch receptor. The attachments of the strand suggested a proprioceptive function characteristic of the strand receptor, and recording afferent spikes when moving the whole coxa shows a response to low and high angular velocities (Fig. 4*b*). This information may be used by the CNS for position control in still animals and for monitoring angular velocities of the moving coxa, as stretching the receptor strand causes increased motoneurone discharge of the coxal depressor muscles (Fig. 4*c*). Contractions of these muscles counteract forces that elongate the strand.

The metathoracic receptor does not seem to be the only one of this new morphological type in locusts. Peripheral cobalt stainings showed similar structures in a comparable position in the fore-and middle leg and others spanning the coxa–trochanter joints of all three leg pairs.

All previously described insect mechanoreceptors have their sensory cell bodies in the periphery as most invertebrate sense organs do. Exceptions have been found in some annelid[6] and crustacean[7–9] species, for which sensory neurones with somata in the CNS have been reported. The discovery of this new type of proprioceptor in the locust brings the insects in line with these other invertebrate groups. The location of the sensory structure in the proximal leg joints and their appearance at the light-microscopic level reminds one of certain strand receptors reported for decapod Crustacea[7]. However, there is a striking difference in signal propagation: locust organs send their information to the CNS by means of action potentials, whereas crustacean receptors only transmit graded potentials[8,9].

The ontogeny of the described sensory cells remains an unsolved problem: do they develop like insect mechanoreceptor cells from late ectodermal cells during embryogenesis with cell bodies migrating into the CNS or are they derived from neuroblasts like motoneurones and interneurones?

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Dopamine depolarisation of mammalian primary afferent neurones

Joel P. Gallagher, Hiroe Inokuchi* & Patricia Shinnick-Gallagher

Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, Texas 77550

Dopamine (DA) is an important neurotransmitter or neuromodulator in the mammalian nervous system. As such, it is implicated in the aetiology and therapy of various disease conditions—for example, Parkinson's disease, schizophrenia, Huntington's disease and tardive dyskinesia. However, only limited electrophysiological information is presently available concerning dopamine receptors in the mammalian nervous system[1], and there are only three reports[2–4] in which intracellular techniques have successfully recorded the action of DA on individual central neurones. In all cases, DA depolarised the respective neurones. In the periphery, DA is reported to hyperpolarise superior cervical ganglia[5–9]. However, this hyperpolarisation has been shown to be due to activation of α-adrenoreceptors and not to a response of DA on a DA receptor[8,9]. Peripheral DA actions have also been described presynaptically[10,11], but are difficult to study electrophysiologically for technical reasons. As a result, little is known at the membrane level about the effects of drugs thought to modulate or interact with DA receptors. In the present report, we describe a depolarising action for DA on the cat dorsal root ganglion.

Lower lumbar dorsal root ganglia (DRG) were dissected from adult cats (2–5 kg) that were anaesthetised intraperitoneally (i.p.) with a mixture of α-chloralose (60 mg kg^{-1})) and pentobarbital (5 mg kg^{-1}). The isolated ganglia were maintained at 37 °C in physiological solution aerated with 95% O_2 and 5% CO_2. A physiological solution of the following composition was used to superfuse the preparation (in mM): NaCl, 117; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25 and glucose, 11.5. Standard intracellular recording techniques[12] using K-citrate (2 M) filled microelectrodes were used. Known concentrations of drugs were added to the superfusate.

We have been investigating the pharmacology of a γ-aminobutyric acid (GABA) receptor that has been characterised on the cell body of cat dorsal root ganglia[12]. As a result of *in vitro*[12–14] or *in vivo*[15] techniques with ganglia from adult animals, it has been assumed that receptors on the somata of primary afferent neurones may be similar to those on their terminals. There have been two reports[16,17] which suggest that neuroleptic drugs (agents thought to interact with central DA receptors) may also affect GABA–GABA receptor interactions.

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* Present address: Department of Physiology, Kurume University Medical School, Kurume, Japan.

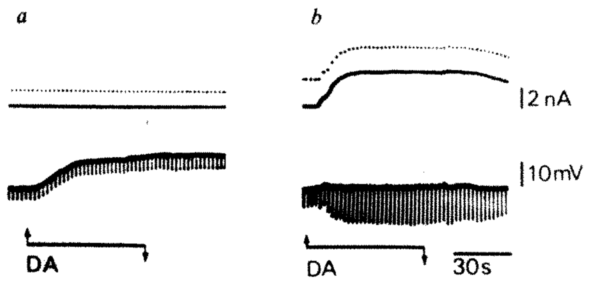

Fig. 1 Effects of bath applied DA (5×10^{-4} M) on membrane potential and resistance. *a*, Upper pair of tracings (current monitor) represents constant hyperpolarising current pulses. Lower tracing (voltage monitor), solid line represents membrane potential from which anelectrotonic responses deflect downward. *b*, Same as *a* except a current is applied manually during the DA application to prevent the drug-induced depolarisation. In these conditions, the membrane resistance increases during DA.

To examine this possibility, we applied the putative DA-antagonists chlorpromazine (CPZ) and haloperidol while monitoring iontophoretically induced GABA depolarisations. Neither CPZ nor haloperidol at concentrations to 10^{-5} M altered the GABA response. However, at 10^{-5} M, haloperidol occasionally depolarised the membrane and would block an action potential induced by cathodal stimulation through the recording electrode. A similar depolarising action for haloperidol had been reported at a DA receptor on a vertebrate neuronal cell hybrid[18]. As a result of this depolarising action by this DA antagonist, we undertook the following experiments to investigate whether DA has a direct action on these cells.

After impaling a neurone its effective membrane resistance (1/conductance) was determined from the ratio of voltage/current measurements. Electrotonic potentials required for the above measurements were generated by brief repetitive anodal current pulses delivered through the recording electrode. When DA was applied to the preparation, a sustained membrane depolarisation with little conductance change was recorded (Fig. 1*a*). Because DA depolarised the membrane at a slow rate, the membrane potential could be held constant during the DA application by passing a continuous hyperpolarising current through the recording electrode. In these 'manual clamp' conditions[19], DA decreased conductance (Fig. 1*b*). In one other report[3], the DA depolarisation was usually not associated with a conductance change. The lack of a conductance change during the unclamped dopamine response is probably due to the membrane rectifying properties of the dorsal root neurones. Because of the rectifying properties we intend to determine the ionic mechanism of this DA-induced depolarisation using voltage clamp techniques. Preliminary results show that unlike the GABA-induced depolarisation chloride is not involved in the DA-induced depolarisation, whereas a decreased potassium conductance is, at least, contributory.

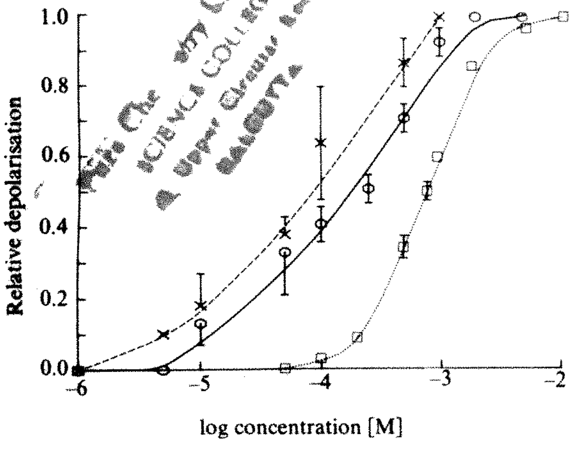

Fig. 2 log concentration–response relationships for DA ($K_a = 1.3\times10^{-4}$ M), apomorphine ($K_a = 7\times10^{-5}$ M) and GABA ($K_a = 7.8\times10^{-4}$ M). ×, Apomorphine; ○, dopamine; □, GABA.

Dopamine, at various concentrations, produced similar slow membrane depolarisations in 75% of the cells to which it was applied (209/275). In no instance did DA hyperpolarise the membrane. The complete concentration–response relationships for DA from 13 neurones are depicted in Fig. 2. For comparison, various concentrations of apomorphine ($n = 4$), a specific DA agonist, and GABA ($n = 10$) were also applied to the preparation. Apomorphine ($ED_{50} = 7\times10^{-5}$ M) was at least twice as effective as DA ($ED_{50} = 1.3\times10^{-4}$ M) in causing a membrane depolarisation. The maximum depolarisation produced by DA and apomorphine was about 10 mV. Both DA and apomorphine produced sustained, non-desensitising depolarisations for as long as the drug was in contact with the preparation. This action differed from one observed when GABA is applied for a prolonged period because the GABA depolarisation rapidly declines as the receptor becomes desensitised[12,13].

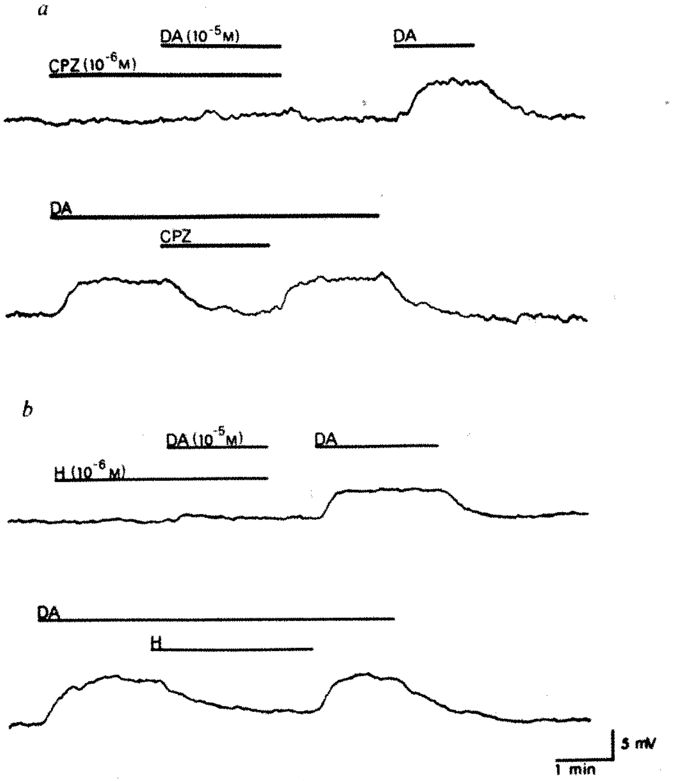

Fig. 3 Effects of antagonists on dopamine depolarisation. *a*, Chlorpromazine (CPZ) effects on membrane potential and on DA-induced depolarisation. *b*, Haloperidol (H) on membrane potential and on DA-induced depolarisation.

Of the drugs tested, only chlorpromazine (CPZ) and haloperidol antagonised the action of DA (Fig. 3). CPZ (10^{-6} M) had no effect on the resting membrane potential but antagonised the depolarising action of DA (Fig. 3*a*). In the continued presence of DA, the addition of CPZ inhibited a DA depolarisation. Haloperidol (10^{-6} M) produced a similar antagonistic action (Fig. 3*b*).

Other amines were applied to the sensory ganglion by the superfusion technique. Although noradrenaline depolarised the ganglion, it did not depolarise to the same degree as DA, even at concentrations 10 times greater than that of DA. The depolarisation induced by noradrenaline could not be antagonised by phentolamine, an α-adrenoreceptor antagonist. Propanolol, a β-adrenoreceptor antagonist was also ineffective in blocking the action of the amines. Furthermore, adrenaline and serotonin were less effective depolarising agents than noradrenaline. This weak effect of serotonin was unexpected as it has been shown to be active at the nodose ganglion[20] and the superior cervical ganglion[21]. Isoprenaline did not depolarise.

The concentrations of DA ($ED_{50} = 1.3 \times 10^{-4}$ M) required to depolarise the ganglion are high. Nonetheless, we believe that this action is specific because the responses are antagonised by classical DA antagonists and not affected by the α- or β-adrenoreceptor antagonists. In a recent study with isolated toad spinal cords, catecholamines, including DA, at concentrations similar to those used here, depolarised dorsal root terminals[22]. In addition, there is evidence that dopaminergic neurones are present in the mammalian spinal cord[23]. These authors have described a differential distribution for DA within the cord; DA concentration was found to be two to three times higher in the dorsal horn than in the ventral horn. This distribution pattern would support the fact that DA receptors on the sensory ganglion soma may also be present on their terminals. At present, there is no published evidence regarding DA binding to dorsal root ganglia.

The dorsal root ganglion may provide us with essential information about the interaction of DA with its receptor and of drugs thought to alter its action at the membrane level. With this knowledge a better insight into the therapeutic approach to various diseases of the central nervous system may be achieved.

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Serotonergic neurones are not involved in action of L-dopa in Parkinson's disease

Eldad Melamed, Franz Hefti, James Liebman, Arthur J. Schlosberg & Richard J. Wurtman

Laboratory of Neuroendocrine Regulation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Under normal circumstances, L-dopa is converted to dopamine (DA) in DA-containing neurones of the corpus striatum by the enzyme aromatic L-amino acid decarboxylase[1]. Although L-dopa has been used widely to treat Parkinson's disease, the site of its decarboxylation to DA in the parkinsonian striatum is unknown. It was initially assumed that dopa administration enhances DA synthesis and release in surviving nigrostriatal neurones[2–6]. Subsequent *in vitro* studies provided evidence that exogenous dopa might also be taken up, decarboxylated[7–9], and released as DA[8,10] by serotonergic terminals, suggesting that non-dopaminergic neurones also may mediate some of dopa's therapeutic effects[10]. During long-term dopa treatment in parkinsonism, its efficacy often declines[11], and diurnal fluctuations in its effectiveness (the 'on-off' phenomenon) can become manifest in previously responsive patients[12]. If the effects of dopa depend solely on surviving dopaminergic neurones, its decreasing efficacy with time could imply continuing degeneration of striatal terminals[11,13]. However, if exogenous dopa can also affect striatal DA transmission by being decarboxylated in serotonergic neurones, the declining therapeutic efficacy could reflect involvement of serotonergic neurones in the pathology of Parkinson's disease; this has previously been reported, but remains controversial[14–17]. Therefore, we examined the decarboxylation of exogenous dopa in an animal model of parkinsonism—rats with unilateral nigrostriatal lesions[18]—which were further subjected to lesions of the striatal serotonergic projections. Our data indicate that combined destruction of striatal dopaminergic and serotonergic terminals has little additional effect, beyond destroying dopaminergic neurones, on dopa's ability to enhance striatal DA release and do not favour the concept that serotonergic neurones are involved in mediating the therapeutic effects of L-dopa.

Groups of 5–10 Sprague–Dawley rats (200–250 g; Charles River) had free access to water and Big Red rat chow (Charles River). Lesions in midbrain dorsal and median raphe nuclei were produced electrolytically (2 mA, 20 s in the dorsal and 2 mA, 25 s in the median raphe nucleus). One month later, unilateral nigrostriatal lesions were performed in raphe-lesioned and control rats by injecting stereotaxically 8 μg 6-hydroxydopamine (OHDA) in 4 μl saline containing 0.2 mg ml^{-1} ascorbic acid into the right anteromedial substantia nigra (level A 2400, according to König and Klippel[19]). This was immediately followed by an additional injection of 4 μg in 2 μl into the right medial forebrain bundle (level A 4000) to increase the lesion's severity. Two weeks after the OHDA treatments, animals were injected with L-dopa (100 mg per kg, intraperitoneally), placed in metal cylinders (35 cm diameter), and their turnings (full circles) were counted during the 5-min interval between 40 and 45 min after the injection. Rats were then decapitated and both striata were dissected separately and frozen on dry ice. Tissues were homogenised in ice-cold distilled water; buffered aliquots were assayed for tyrosine hydroxylase (TH)[20] and dopa decarboxylase (DDC) activities[21], and deproteinised aliquots for 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using high-performance liquid chromatography with electrochemical detection[22]. Serotonin in small striatal samples was determined by an enzymatic–isotopic method[23].

Since striatal DA concentrations after dopa administration can no longer indicate the extents of nigrostriatal lesions, we used TH activity for this purpose. TH activities and DA levels were highly correlated ($r = 0.91$) in OHDA-lesioned striata of rats not receiving dopa (F.H. *et al.*, in preparation). After OHDA injections, there were marked reductions (by 90–95% compared to control or contralateral striata) in TH activity in lesioned striata, indicating severe unilateral destruction of the nigrostriatal system in all groups of animals (Table 1). The raphe lesions produced marked reductions (by approximately 90%) in serotonin levels in both OHDA-lesioned and contralateral striata (0.75 ± 0.15 and 0.55 ± 0.13 ng per mg protein, respectively; mean ± s.e.m.), compared with levels in control striata (6.68 ± 1.18 ng per mg protein). Although dopa administration has been reported to decrease brain serotonin levels[24], we found no decrease in another group of unlesioned, dopa-treated animals (6.82 ± 0.52 ng per mg protein, $n = 10$). DDC activity was reduced in OHDA-lesioned striata by 76–87% compared with

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those in control and contralateral unlesioned striatal tissues. Despite severe destruction of the nigrostriatal system, DDC activity was relatively less reduced than TH activity, confirming that striatal DDC exists both within and outside dopaminergic neurones[25]. DDC activity in striata of animals with unilateral nigrostriatal lesions was not decreased further if animals were subjected to raphe lesions. Dopa administration caused no significant changes in TH and DDC activities in lesioned or control striata.

Nigrostriatal lesions produced marked ipsilateral depletions of striatal DOPAC and HVA (Table 1). Dopa administration increased the concentrations of these DA metabolites in unlesioned, contralateral striata. It also markedly elevated DOPAC and HVA levels in lesioned striata, indicating that decarboxylation of exogenous dopa occurs despite a marked reduction in the number of DA-containing nerve endings. In rats with combined raphe-nigral lesions, the increases in striatal DOPAC and HVA after dopa administration were similar to those observed in animals with unilateral nigrostriatal lesions alone. This indicates that dopa decarboxylation in striata containing a diminished number of dopaminergic terminals is not altered after additional destruction of the striatal serotonin input.

Dopa reportedly increases locomotor activity in rats, and this action is further amplified after destruction of central catecholaminergic neurones by intraventricular OHDA[26]. If brain serotonergic neurones were also destroyed, dopa's effects on motor activity were either reduced[27] or unchanged[28]. In rats with unilateral nigrostriatal lesions, dopa administration induces contraversive circling behaviour, attributed to enhanced DA synthesis and release within the lesioned striatum[18]. In the present study, dopa-treated rats with combined raphe-nigral lesions displayed vigorous turning away from the lesioned side; this behaviour did not differ from circling noted in rats with unilateral nigrostriatal lesions alone (Table 1). This finding contradicts a recent report[29] showing diminished rotational response to L-dopa when both striatal serotonergic and dopaminergic neurones have been destroyed unilaterally. In this report, the unilateral lesioning of serotonergic neurones (by injecting 5,7-dihydroxytryptamine into one lateral ventricle), probably caused an additional asymmetry between the two striata, which by itself could have affected turning behaviour[30,31]. In our study, raphe lesions reduced serotonin levels by about the same extent in both striata; such lesions do not affect the circling associated with asymmetries in the nigrostriatal system[32,33].

To examine the relative contribution of DDC within serotonergic neurones to total striatal DDC activity, rats were subjected to electrolytic lesions in dorsal or median raphe nuclei, or in both nuclei. Dorsal and combined dorsal-median raphe lesions reduced striatal serotonin content (Fig. 1), confirming the presence of a projection from the dorsal raphe to the striatum in the rat[34,35]. Striatal DDC activity (measured using [1-^{14}C]dopa as substrate) did not decrease despite marked reductions in serotonin. This finding is consistent with our previous observation (Table 1) that a raphe lesion does not further reduce striatal DDC activity in rats with nigrostriatal lesions. These data suggest that the decarboxylase contained within serotonergic neurones contributes insignificantly to the total DDC activity of the striatum.

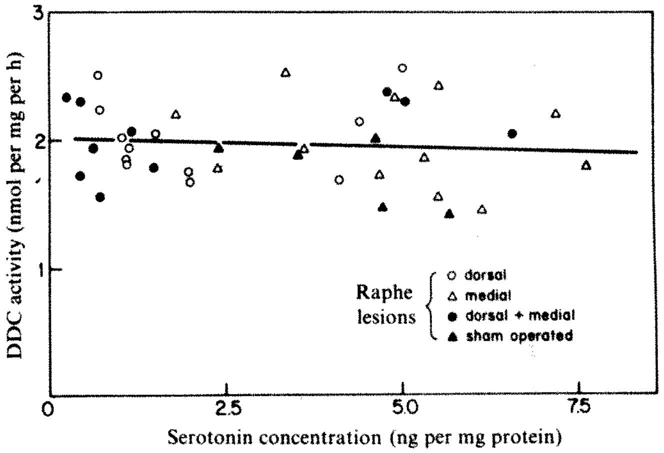

Fig. 1 Striatal dopa decarboxylase activity and serotonin concentrations in rats with electrolytic lesions in the midbrain dorsal or median raphe nuclei, or in both nuclei. Sham-operated rats were used as controls. Rats were killed 2 weeks after the lesions were produced, and the right and left striata from each rat were pooled and analysed for dopa decarboxylase activity (using [1-^{14}C]dopa as substrate)[21] and for serotonin[23]. Regression line was calculated by the least squares method; $r = -0.10$.

Our study shows that in rats with severely damaged nigrostriatal systems, an additional lesion destroying striatal serotonergic neurones does not alter the ability of exogenous dopa to enhance DA release or to cause a behaviour (contraversive circling) thought to be induced by DA release. Hence, in our experimental conditions, dopa's effects possibly are not significantly dependent on its uptake in, decarboxylation in, or

Table 1 Effect of dopa on striatal DOPAC and HVA concentrations in rats with combined raphe and unilateral nigrostriatal lesions

	TH	DDC	DOPAC	HVA	Turning
	(nmol per mg per h)		(ng per mg)		(turns per 5 min)*
Controls	0.153±0.010	2.89±0.23	0.58±0.10	0.64±0.06	0
OHDA lesion:					
OHDA-lesioned	0.016±0.008†	0.42±0.05†	0.02±0.02†	0.04±0.01†	
Contralateral	0.148±0.007	2.24±0.21	0.43±0.06	0.43±0.04	
OHDA lesion + DOPA					
OHDA-lesioned	0.008±0.004†	0.49±0.12†	1.77±0.25‡	1.40±0.20‡	20.5±6.6
Contralateral	0.154±0.017	2.11±0.21	2.65±0.53§	2.05±0.39§	
OHDA + raphe lesions + DOPA					
OHDA-lesioned	0.007±0.001†	0.56±0.09†	1.34±0.76‡	1.40±0.62‡	31.0±7.0
Contralateral	0.128±0.009	2.95±0.73	3.65±1.55§	2.37±0.84§	

Values are means ± s.e.m. Rats were lesioned electrolytically in the dorsal and median raphe nuclei. One month later, these animals and other control rats were injected unilaterally with 8 μg OHDA into the substantia nigra and 4 μg into the medial forebrain bundle. Two weeks after the OHDA lesions were produced, animals received 100 mg per kg L-dopa intraperitoneally and were put in metal cylinders for measurement of turning behaviour between 40 and 45 min after injection. Rats were then decapitated and the enzyme activities (TH and DDC) and DOPAC and HVA concentrations were measured in both striata. Data were subjected to analysis of variance followed by Scheffe's test.

* Full circling away from the OHDA-lesioned side.

† Different from controls and from contralateral side by $P < 0.01$.

‡ Different from lesioned striatum of untreated rats by $P < 0.01$.

§ Different from controls and from contralateral side of lesioned untreated rats by $P < 0.05$.

release as DA from striatal serotonergic terminals[8-10]. Thus our findings do not suggest a major role for serotonergic neurones in mediating dopa's effects in Parkinson's disease. In intact striata, DDC activity is localised predominantly in DA-containing terminals (Table 1). The DDC activity remaining after nigrostriatal lesions could be contained within surviving DA-containing neurones and in other striatal constituents, such as the capillaries[36,37]. DA formed from dopa by DDC within capillary endothelium probably lacks functional significance, since it may not be able to traverse the blood–brain barrier and reach striatal synapses[36,38,39]. The rotational behaviour[40] and striatal DA formation (E.M. *et al.*, in preparation) after dopa administration are not abolished when rats with unilateral nigrostriatal lesions are given a drug that blocks vascular DDC, indicating that, in lesioned rats, dopa is also converted to DA outside striatal capillaries. Further studies should determine if this decarboxylation occurs exclusively within surviving dopaminergic neurones or also in other striatal compartments.

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Black widow spider toxin-induced calcium fluxes and transmitter release in a neurosecretory cell line

A. Grasso, S. Alemà, S. Rufini & M. I. Senni

Laboratory of Cell Biology, CNR, Via Romagnosi 18/A,
00196 Rome, Italy

Several polypeptide neurotoxins affect presynaptic functions by interfering with chemical neurotransmission. This group of toxins includes botulinum toxin[1], tetanus toxin[2], β-bungarotoxin[3] and black widow spider toxin (BWSTx)[4-6]. While the effect of the first three toxins is mainly a rapid and severe block of neurotransmitter release, BWSTx affects transmission by a massive stimulation of mediator release[4-6]. Despite various hypotheses put forward to explain the action of BWSTx at the level of nerve terminals, there is still a considerable degree of uncertainty as to the cation dependence of venom action[7-11]. Study of the toxin mode of action at the biochemical level has been hampered by the complexity and cellular heterogeneity of the preparations used, neuromuscular junction[10] or synaptosomes[6]. PC12 cell line, derived from a rat phaeochromocytoma[12], seems to be an excellent model in view of its property of synthesising and storing noradrenaline, dopamine and acetylcholine, and releasing them in depolarising conditions[13]. We have recently shown that highly purified BWSTx stimulates secretion from PC12 cells of previously taken up radioactive dopamine (DA) and noradrenaline (NA) (ref. 14 and manuscript in preparation). We report here that the earliest detectable event after toxin treatment of such cells is a massive increase of cytosolic calcium.

When PC12 cells grown in monolayer and loaded with ^{3}H-labelled DA are exposed to BWSTx in a nominally Ca^{2+}-free medium, containing 1 mM EGTA, the radioactivity released in the medium equals that of untreated controls (less than 1% of total intracellular radioactivity per min). Extensive washing of the cells and substitution of the latter medium with a Ca^{2+}-containing medium (1.8 mM) results in a release of more than 30% of total labelled catecholamines in the following 5 min (Fig. 1). The amount of transmitter release is quantitatively comparable with that obtained with an equal amount of toxin added to a Ca^{2+}-containing medium (dashed line, Fig. 1). BWSTx-induced release of ^{3}H-DA is completely blocked if cells are pre-exposed to micromolar concentrations of concanavalin A or if toxin is challenged with monospecific antibodies before addition to cultures. However, if lectin or antibodies are added after toxin is bound to cell membrane in EGTA, Ca^{2+}-triggered effect (see Fig. 1) is maintained (data not shown). Furthermore Ca^{2+}-dependent release of DA after toxin treatment is temperature-dependent, being reduced by over 80% when temperature is lowered from 37° to 8 °C.

Table 1 Effect of several agents on Ca^{2+} flux and dopamine release induced by BWSTx and K^+

	Percentage inhibition of maximum effect			
	^{45}Ca flux		DA release	
Condition	BWSTx	K^+	BWSTx	K^+
TTX (1×10^{-6} M)	0	0	0	0
Sea anemone Tx II (5×10^{-6} M)	0	0	0	0
Co^{2+} (1×10^{-3} M)	0	80	0	75
(2×10^{-3} M)	35	98	30	95
Cd^{2+} (2×10^{-3} M)	ND	ND	0	90
Verapamil (5×10^{-5} M)	32	70	10	75
Concanavalin A (5×10^{-7} M)	100	0	100	0

Experimental conditions were as described in Fig. 2, except that BWSTx (8 nM) and K^+ (56 mM) were directly added to cultures in standard HR medium (1.8 mM $CaCl_2$). ^{45}Ca uptake and ^{3}H-DA release were measured after 6 min incubation at 25 °C. Each value represents the mean of duplicate assays in a typical experiment. Values are expressed as a per cent of maximum effect in control treated cultures. ND, not determined.

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It thus seems that BWSTx binds to PC12 membrane in the absence of Ca^{2+} but is unable to induce catecolamine secretion unless physiological concentrations of divalent cations are added to the medium. The next question which arises concerns the site at which external calcium ions are necessary to make the toxin effect apparent. Is the toxin directly activated by Ca^{2+} ions or does it induce transmitter release by allowing Ca^{2+} ions to enter the cells? To test the latter question we made use of PC12 cells exposed to toxin in a medium containing EGTA. After 10 min unbound toxin was extensively washed out and the cells quickly exposed to a medium containing 1.8 mM ^{45}Ca. As shown in Fig. 2*a*, immediately after the addition of the Ca^{2+}-Ringer a massive influx of ^{45}Ca is measurable in cells pretreated with a saturating concentration of toxin (7 nM). This influx is several times higher than that induced by depolarising concentrations of K^+ or by 0.7 nM toxin. In the latter case net stimulation of ^{45}Ca uptake tends to level off after about 100–150 s. Release of ^3H-DA was followed in conditions identical to those used for the Ca^{2+} flux experiments (Fig. 2*b*). Stimulation of DA release over background is very rapid and its time course seems to be concomitant with that of ^{45}Ca influx at both toxin concentrations. Interestingly, although the uptake of ^{45}Ca elicited by 7 nM toxin is 10- to 12-fold higher than that due to 0.7 nM toxin (or 56 mM K^+), the ratio, as far as DA secretion is concerned, is only 2.5–3.0. This suggests that only a proportion of the Ca^{2+} entering cells is saturating those intracellular sites involved in secretion, the rest being perhaps responsible for the cellular swelling observed as a consequence of long-term exposure to high toxin concentrations (unpublished results). Note that while these results suggest initial Ca^{2+} entrance as a primary event in the induced release, a secondary mobilisation of internal stores of Ca^{2+} is not to be excluded.

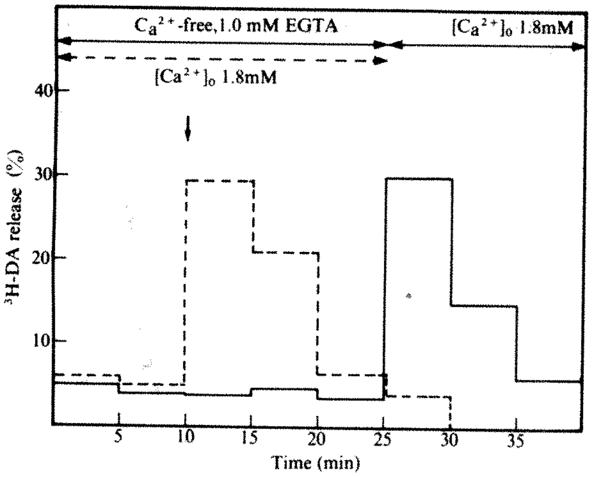

Fig. 1 Influence of Ca^{2+} on dopamine release in response to BWSTx. PC12 cells were grown as monolayer on collagen-coated Petri dishes (35 mm) at a density of 10^6 cells per dish. Cells were washed free of culture medium and incubated at 37 °C for 60 min in 1 ml of HEPES–Ringer (ref. 13) (HR) supplemented with 1 μCi per dish ^3H-DA, 10^{-4} M pargyline and 0.5 mg BSA ml^{-1}. Specific activity of ^3H-DA (Amersham) was 6–7 Ci $mmol^{-1}$. At the end of the uptake period, cultures were brought to 25 °C, washed three times with HR (containing 1.8 mM $CaCl_2$). Sister cultures were washed with a modified Ca^{2+}-free HR buffer (also containing 1 mM EGTA). Cultures were then incubated for 5-min periods with 1-ml aliquots of the respective buffers. The releasing medium was collected from the dishes by careful suction directly into scintillation vials. After two 5-min intervals, experiment was started (arrow) by addition of 5 pmol of purified BWSTx (molecular weight 125,000)[6]. Toxin was present only in the following 5-min interval. Dashed line refers to cultures in the presence of HR. Continuous line refers to cultures in EGTA-HR. In this set, Ca^{2+}-containing HR was replaced after three EGTA-HR washes following toxin addition. At the end of the experiment cells were solubilised with 2% SDS. Radioactivity was counted in 5 volumes of Aquasol-2. ^3H-DA release is expressed as a per cent of total radioactivity present in the cells at the beginning of the experiment. The cells remain firmly attached to the substrate during the whole experiment.

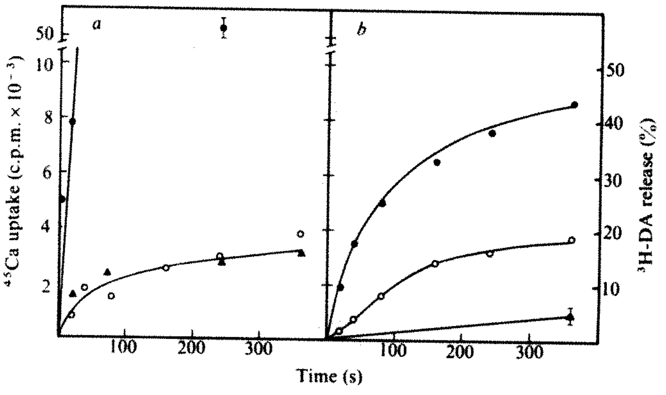

Fig. 2 Time course of ^{45}Ca uptake and ^3H-DA release induced by two different toxin concentrations. *a*, PC12 cells were grown in conditions identical to those described in Fig. 1. Cultures, after washing with EGTA-HR, were incubated for 10 min at 25 °C with the same buffer but containing, when appropriate, 0.7 (○) and 7.0 (●) nM BWSTx and bovine serum albumin 0.5 mg ml^{-1}. The medium containing toxin was then removed and cultures challenged with 1 ml of HR containing 1.8 mM ^{45}Ca (5×10^6 c.p.m. ml^{-1}) for the indicated time intervals. 56 mM KCl was present in K^+-stimulation experiments (▲). Uptake was terminated by washing the dishes quickly three times with HR and then solubilising the cells in 2% SDS. Background Ca^{2+} uptake (0.05–0.1 nmol per min per 10^6 cells) was substracted from total counts to obtain the values plotted above. Possible exchange of Ca^{2+} during the course of our measurements was ignored. *b*, PC12 cells preloaded at 37 °C with ^3H-DA as described in Fig. 1, were equilibrated at 25 °C in EGTA-HR also containing the same toxin concentrations as in (*a*) for 10 min. After removal of toxin release was started by addition of standard HR. Background release of tritiated DA (less than 1% per min) is given (▲). DA release expressed as in Fig. 1. Each point represents the mean from three different experiments. Net Ca^{2+} fluxes of comparable magnitude were observed also if BWSTx was added in a standard HR medium. However kinetics were somewhat modified because of toxin diffusion and binding to cells. BWSTx did not cause uptake of ^{45}Ca by non-secretory cells such as normal or transformed chick fibroblasts and rat myoblasts.

Two possible mechanisms by which BWSTx might enable Ca^{2+} to enter the cells are: (1) activation of physiologically operating voltage-dependent Ca^{2+} channels, which have been recently described by Stallcup in PC12 cells[15]; or (2) induction of new channels somewhat analogous to those formed by toxin on artificial lipid bilayers[16]. We found that in agreement with previous findings, both transition metals and verapamil (a calcium-channel blocker) fully inhibit K^+-induced Ca^{2+} influx as well as DA release[17,18] (Table 1). Such full inhibition was not observed when the same compounds were tested on toxin, thus suggesting that the major part of BWSTx effect does not involve direct activation of voltage-dependent Ca^{2+} channels. The moderate reduction in Ca^{2+} entry (and hence in DA release) observed at high Co^{2+} concentrations might be due to Co^{2+} permeating in place of ^{45}Ca, but being unable to stimulate release (see ref. 18). An alternative explanation might involve BWSTx acting through two distinct mechanisms—induction of 'specific' channels and a secondary contribution from Co^{2+} and verapamil-sensitive channels, perhaps activated by a concomitant depolarisation. However, support for the idea that Ca^{2+} entry elicited by toxin is unlikely to involve voltage-dependent channels comes from the observation that, at variance with potassium[18], transmitter release induced by the toxin is not inhibited by high Ca^{2+} concentrations (10 mM) (data not shown). Finally, ^{45}Ca flux induced by BWSTx does not require the presence of Na^+, being enhanced by a factor of two, possibly through a mechanism discussed earlier[15], in a Na^+-free-sucrose medium; even dopamine release is not appreciably affected in the same experimental conditions.

The main result of our experiments is the demonstration that BWSTx produces a rapid and massive influx of Ca^{2+} ions in a toxin-responsive, neurosecretory cell. The main consequence of Ca^{2+} influx is the release of neurotransmitters presumably through a typical exocytotic process[19,20]. Although we have no direct evidence that other cations besides Ca^{2+} cross the cell membrane as a consequence of toxin action, in our conditions neither Na^+ nor Mg^{2+} alone seem to effectively substitute for Ca^{2+} in inducing transmitter release. The present work also confirms and extends the previous observations[21,22] on the antagonistic effect of concanavalin A (see Table 1) by showing that the lectin inhibits BWSTx-induced Ca^{2+} uptake. On the basis of the available evidence, it seems reasonable to postulate that binding to a concanavalin A-sensitive 'receptor' is an obligatory step for the formation of the previously discussed Ca^{2+} 'channels'.

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X-ray-induced *in vitro* neoplastic transformation of human diploid cells

Carmia Borek

Departments of Pathology and Radiological Research Laboratory, Department of Radiology, Cancer Center/Institute of Cancer Research, Columbia University College of Physicians and Surgeons, New York, New York 10032

Techniques have recently been developed to identify and score quantitatively neoplastic transformation caused by X rays in cultured cells derived from rodents[1]. Because of their relevance to estimates of human cancer risk, it is clearly desirable to carry out similar experiments with cultured cells of human origin. The present report describes for the first time the neoplastic transformation *in vitro* of human diploid cells by X-ray irradiation into cells which can progress *in vitro* into advanced stages of neoplastic development, namely, to form colonies in agar and give rise to tumours when injected into nude mice.

We used an early passage of skin fibroblast strain KD cells, originally derived from a skin biopsy of an adult woman[2,3]. The cultures were in their fifth cell passage, dating from the original biopsy. Chromosome G banding analysis carried out on the cells on arrival indicated a normal diploid human karyotype. The doubling time of the KD cells as determined by growth curve analysis[4] was 30–32 h.

The cells were routinely maintained in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum and 1% human serum (GIBCO) and incubated at 37 °C. A dose–response curve for cell survival was carried out as described elsewhere[5] (see Fig. 1). The scheme for transformation experiments is described in detail in Fig. 2 legend. All cells were fed twice a week. Four separate experiments have been carried out using antipain[6] or β-oestradiol. In each experiment control cultures included unirradiated, untreated cells as well as cells unirradiated, but treated with antipain or β-oestradiol. These were set up in equal numbers to the experimental flasks and subcultured in the same manner.

Within 2 months of irradiation, discrete foci were detected in the pretreated, irradiated cultures which had been left untouched following the 1:12 split and were now at high density. The foci, consisting of cells with a subtle irregularity in cell to cell orientation, were visible under phase microscopy. Two or three foci were initially detected in each experiment in both antipain- and oestradiol-pretreated, irradiated cultures, suggesting that these compounds were equally effective in potentiating radiation-induced transformation. Within 3 months, 30–40 of these foci per experiment were observed in most of the high-density flasks but not in those which had been given a further subculture (Fig. 2). The latter exhibited these foci after transferring the cells at high density (2×10^6) to 100-mm Petri dishes.

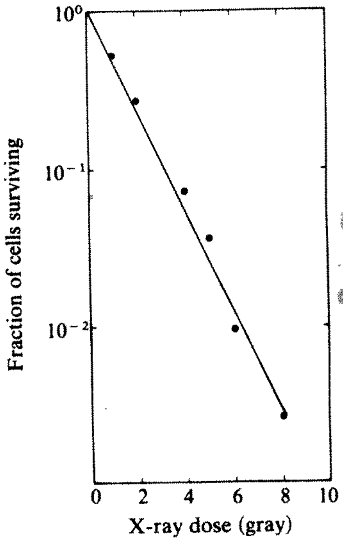

Fig. 1 Cell survival of KD cells following irradiation by X rays, from a Sieman's 300 kVp constant potential generator with an added filter of 0.2 mm copper.

The foci increased in size. A multilayering of cells made them clearly visible to the naked eye (Fig. 3). No foci appeared in the unirradiated cells or in cells treated with antipain or oestradiol alone.

By exchanging the medium for medium containing low calcium concentrations[7], the cells in the morphologically transformed foci underwent an additional, more dramatic morphological alteration (Fig. 3), whereas the normal cells degenerated within 24 h.

Chromosomal G banding analysis carried out on cells isolated from the primary foci showed a human chromosomal constitution with a diploid to near-diploid number of chromosomes

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(range 46–49). A more detailed karyotype will be published elswhere. Saturation density determined on the transformed cells cultured from the foci showed a twofold increase in saturation density over that of the normal. The transformed cells but not the normal parental cells were agglutinable by 25 μg ml^{-1} of concanavalin A (Miles-Yeda).

The ability to proliferate at low serum concentrations (1%) was not confined to the transformed human cells[8] as in rodent systems[1,4], but was also a property of the normal KD cells grown in the same conditions (10^5 cells per 60-mm Petri dish).

Cells isolated from the multilayered foci were seeded in semi-solid agar[9], where they proliferated to form distinct spherical colonies (Fig. 3) with a cloning efficiency of 2–3%. None of the unirradiated KD cells or the irradiated but untransformed cells (seven samples of each) was able to proliferate in agar. Colonies isolated from agar could be recultured in liquid medium or further propagated in agar.

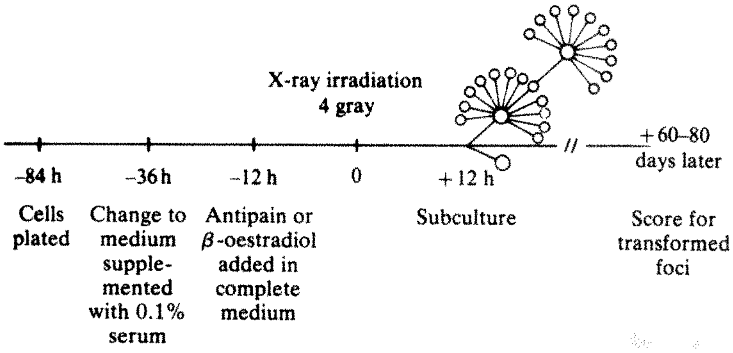
X-ray irradiation
4 gray
−84 h
−36 h
−12 h
0
+12 h
+60–80 days later
Cells plated
Change to medium supplemented with 0.1% serum
Antipain or β-oestradiol added in complete medium
Subculture
Score for transformed foci

Fig. 2 Experimental design for the induction of human cell transformation *in vitro* by 4 gray of X rays (1 gray = 100 rad). Cells were trypsinised (0.25% trypsin), suspended in complete medium and plated in tissue culture flasks (75 cm^2) (Falcon) at 2.6×10^5 cells. Two days later, the medium was replaced with MEM containing 0.1% serum for 24 h, whereby cell proliferation was greatly reduced. After 24 h, medium was again exchanged, this time for complete medium (11% serum) containing either 6 μg ml^{-1} of the protease inhibitor antipain[6] or 1 μg ml^{-1} β-oestradiol (Sigma). Experimental cultures were irradiated 12 h later with 4 gray of X rays as previously described[5]. All cultures were divided into two 10–12 h after irradiation and reseeded in fresh complete MEM without antipain or oestradiol. At near confluency, one of the two flasks was subcultured into 12 flasks, the other being left undisturbed. In each experiment, when these 12 flasks reached confluency, one flask was subcultured 1 : 10 and thus defined as 'continuously passaged', and the remaining 11 flasks were left at high density.

The ultimate proof of the neoplastic nature of the cells transformed *in vitro* is their ability to form tumours in an appropriate host. Swiss *nu/nu* mice were injected intradermally with 5×10^6 cells (suspended in 1 ml medium) into the subscapular region of animals X-ray-irradiated 24 h earlier with 450 rad or unirradiated[10].

Whereas the five transformed lines tested formed colonies in agar, three gave rise within 6 weeks both in the irradiated and unirradiated mice to tumours which have been characterised as noninvasive fibrosarcomas. Cells cultured from the tumours possessed a human karyotype which will be described in detail elsewhere. None of the unirradiated or the irradiated but untransformed cells (seven samples of each) gave rise to tumours in the nude mice.

The present experiments show that human diploid cells can be transformed *in vitro* into tumorogenic cells by X-ray-irradiation. Cultures which were treated and irradiated in the non-dividing state, as well as those which were allowed only four or five cell divisions before reaching confluency (and not subcultured further), did not exhibit transformed foci. This indicates that cell replication is required for the fixation and expression of radiation-induced neoplastic transformation in diploid human cells, and a quantitative assessment is now in progress. This requirement has been shown to exist in the case of rodent cells transformed *in vitro* by radiation[11-13] or chemicals[14,15] and in human cells transformed *in vitro* by chemicals[3]. It was of interest that cells which were not recognisable as transformed at low density expressed transformation when replated at high density in close contact.

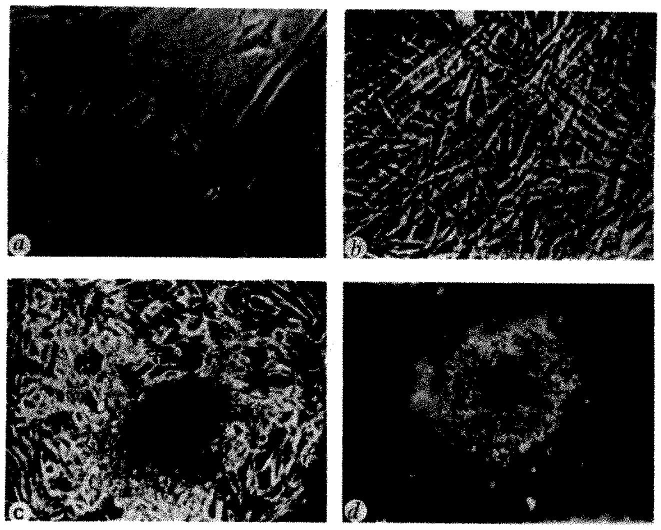

Fig. 3 *a*, Human KD cells in irradiated but untransformed cultures. Phase ×58. *b*, Human KD cells transformed *in vitro* by 4 gray. Note the criss-cross pattern of the cells. Phase ×58. *c*, The same area as in *b* 24 h after incubation in low calcium. Phase ×58. *d*, X-ray-transformed KD cells growing in 0.33% agar. ×12.

The complex experimental design used here takes advantage of the following. (1) The cells which were well adapted to complete medium were subjected to conditions of serum deprivation for a period of 24 h, thus reaching a complete quiescence, indicated in these conditions by specific conformational changes in chromatin (Hoen, personal communication). (2) The addition of complete medium 24 h later led to a synchronous wave of DNA synthesis and treatment of the cells at that point enabled the capturing of cells entering S phase.

We do not know the mechanism of action of oestradiol and antipain in this system. Neither compound exhibited mitogenic activity at the concentration used and both seemed equally effective. We have previously shown that antipain, a protease inhibitor, when present in cultures during irradiation enhanced the yield of X-ray-induced neoplastic transformation in hamster and mouse cells[16]. Oestradiol has been used to potentiate chemically induced transformation[17] and although steroids have many functions, they do have some protease inhibitor activity[18]. Thus, one could speculate that the enhancing action of both compounds may be in part through a common mechanism related to this property.

Although the above experimental regime has been successful for the induction and detection of radiation-induced transformation in the KD cells, it must be stressed that we are relatively ignorant of the best experimental design for inducing transformation and detecting transformants in human cells. Note, for example, that although partial cell synchrony and potentiation of transformation by hormones was a necessary step in the transformation of some diploid human cells by chemical carcinogens[17], it was not required for the induction of transformation in other diploid cells[3]. Because the latter[3] were, in fact, the KD cells, we are now evaluating other experimental designs to induce and detect transformation by radiation in the human cells. The ability to transform human cells *in vitro* by

X-ray irradiation opens up a wide range of studies which should aid in the assessment of human risk to radiation-induced carcinogenesis and in the evaluation of treatments to inhibit its expression and progression.

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Mechanical significance of streptostyly in lizards

Kathleen K. Smith

Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

The morphology of the lizard skull has been a subject of study for more than a century, particularly with respect to the morphology and function of the major jaw adducting muscles[1–5] and the mechanics of the moving parts[6–9]. It is possible that controversies surrounding the extent and timing of bone movement, muscle contraction and force generation will be resolved by techniques such as electromyography, cineradiography and measurement of bone strain[3,10]. I present here data that facilitate a reconsideration of the function of the pterygoideus muscle, one of the two major jaw adducting muscles, and the mechanical significance of movements of the quadrate around the quadrate-squamosal joint. This movement, known as streptostyly[6] occurs in all living lizards[11] and also characterises the earliest members of the order[12–14]. On the basis of my data I propose that streptostyly in lizards is a means by which the mechanical advantage of the pterygoideus muscle is increased, so that this muscle makes a major contribution to bite force.

The data were gathered from simultaneous electromyographic and cineradiographic experiments on *Varanus exanthematicus, Ctenosaura similis*, and *Tupinambis nigrapunctata*, cineradiographic experiments on *Uromastix aegyptius*, and strain gauge and force transducer recording of biting in *V. exanthematicus*. Materials and methods were essentially the same as before for both cineradiography and electromyography[10] and for strain gauge experiments[11]. (Full details of the experiments will be reported elsewhere.)

Figure 1 shows the major morphological features to be discussed here. Of the three major jaw adducting muscles in lizards, the pterygoideus in particular has several interesting features. It is large; in *Varanus*, for example, it is 30–40% of the total adductor mass by weight; however, its mechanical advantage around the quadrate-mandibular joint is small. Generally it is not emphasised as an effective force producing muscle[3,7–9] or it is assumed to be important only in initiating fast closing[4,5]. Its position is such that in addition to any effect at the quadrate-mandibular joint, contraction will draw the lower end of the quadrate forward (protraction) relative to the quadrate-squamosal joint.

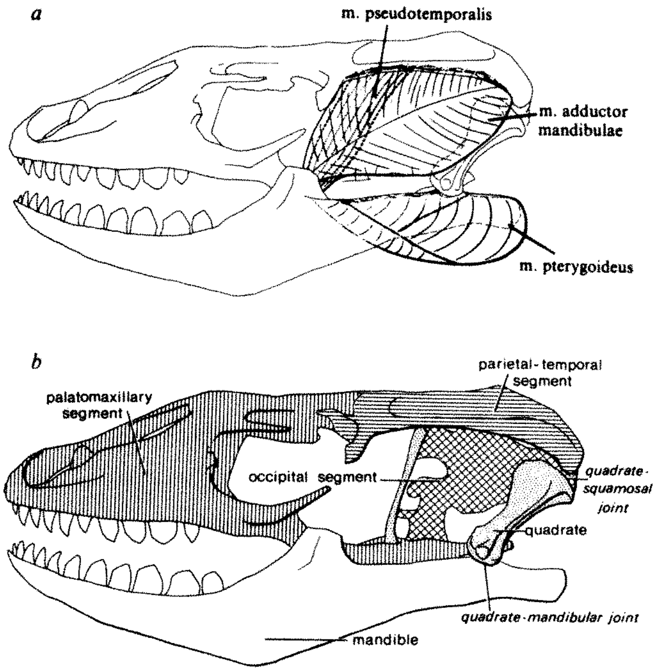

Fig. 1 *a*, Diagram of jaw adducting muscles in *Varanus exanthematicus*. The highly pinnate m. adductor mandibulae originates on the parietal-temporal and occipital units and inserts by means of a large central tendon on the coronoid region of the lower jaw. The m. pseudotemporalis originates from the anterior portions of the parietal-temporal and occipital units and inserts on the coronoid region medial to the m. adductor mandibulae. The m. pterygoideus originates on the pterygoid bone and passes ventrally and dorsally to insert on the ventral surface and retroarticular process of the mandible. This muscle, like the m. adductor mandibulae is highly pinnate, with many fibres running from the external aponeurosis of this muscle to the retroarticular process of the mandible. (For details see refs 1 and 2.) *b*, Bony structure of the lizard skull. The segments potentially important in lizard kinesis are indicated. Movement of the palato-maxillary unit relative to the parietal-temporal and occipital is termed mesokinesis, and that of the occipital unit relative to the parietal-temporal and palato-maxillary is termed metakinesis[6]. Movement of the quadrate at the quadrate-squamosal joint is termed streptostyly[6]. See text for further details.

Electromyographic recordings of the pterygoideus muscle show that it is active during all stages of feeding, including both ingestion cycles and 'power phases' when the animal is biting hard on food. Because it has been suggested that the pterygoideus is ineffective as a force-producing muscle, bite force was measured by stimulating this muscle electrically in an anaesthetised animal. The recordings indicated that the pterygoideus can produce a bite force at least equal to that of the adductor mandibulae externus.

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Previous studies of streptostyly[3,7–9,12,14] have, for the most part, assumed that it is most important during jaw opening and closing in cycles of ingestion and that its primary function is to produce anterior movement of the lower jaw. Further, movement of the quadrate is thought to be linked to movement of the maxillary segment.

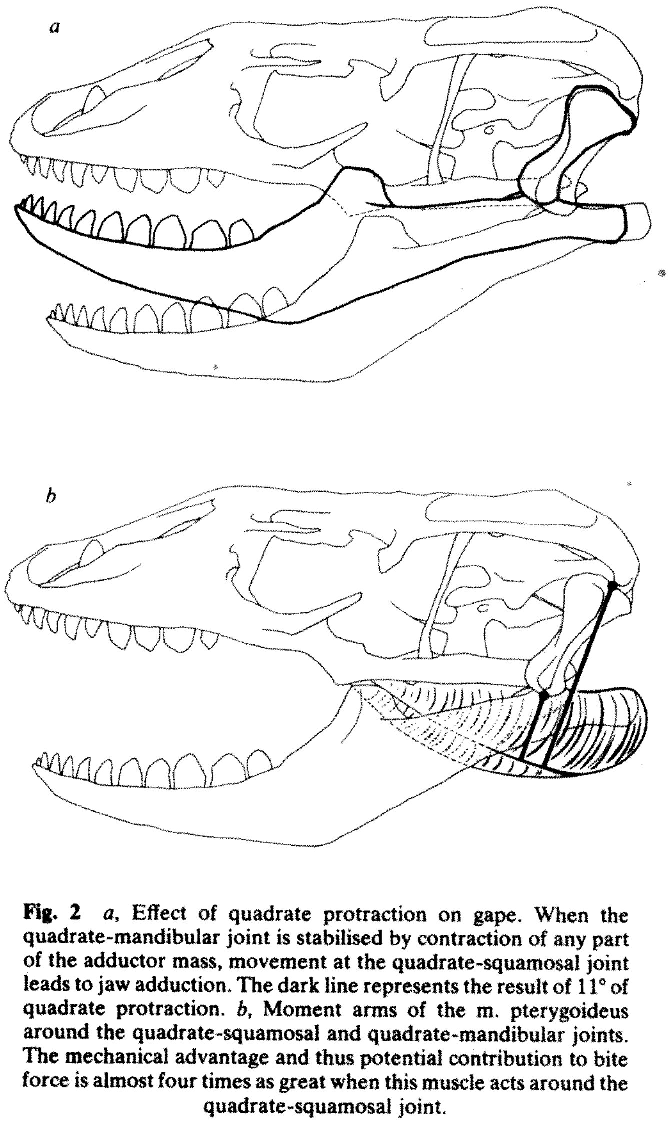

Fig. 2 *a*, Effect of quadrate protraction on gape. When the quadrate-mandibular joint is stabilised by contraction of any part of the adductor mass, movement at the quadrate-squamosal joint leads to jaw adduction. The dark line represents the result of 11° of quadrate protraction. *b*, Moment arms of the m. pterygoideus around the quadrate-squamosal and quadrate-mandibular joints. The mechanical advantage and thus potential contribution to bite force is almost four times as great when this muscle acts around the quadrate-squamosal joint.

Although the quadrate is potentially mobile in all the animals studied, streptostyly was observed only irregularly during cycles of jaw opening and closing in most animals. It was most regular in *Uromastix*. Streptostyly is independent of movement of the palato-maxillary segment (mesokinesis), as Throckmorton[3] found. This would be predicted by careful morphological examination of fresh specimens. In lizards, a ligament binding the quadrate to the pterygoid bone restricts posterior movement of the quadrate, but no such ligament restricts forward movement. Streptostylic quadrates are found in fossil lizards before the appearance of mesokinesis[12–14] and occur in animals with limited mesokinetic potential such as *Uromastix* and *Ctenosaura*. Streptostyly thus preceded and is independent of mesokinesis.

As an alternative to looking at the effect of streptostyly only when the jaw is opening, I note that protraction of the quadrate will close the jaws when the quadrate-mandibular joint is stabilised (Fig 2*a*). Contraction of any of the jaw adductors will lead to such stabilisation. In lizards, therefore, both the quadrate-mandibular and quadrate-squamosal joints are axes for jaw adduction. When an animal bites against resistance, little movement occurs at either joint, but both joints act as fulcra for the production of bite force. I suggest that streptostyly is not primarily important for the forward movement it imparts to the lower jaw, but for its contribution to jaw adduction during hard biting.

As pointed out above, contraction of the pterygoideus muscle leads to quadrate protraction. Figure 2*b* compares the mechanical advantage of the pterygoideus muscle around the quadrate-squamosal and quadrate-mandibular joints. Whereas the adductor mandibulae externus may produce power at the quadrate-mandibular joint, the pterygoideus muscle has primary mechanical advantage to act around the fulcrum at the quadrate-squamosal joint. Thus the significance of the quadrate-squamosal joint is that it allows a muscle with small mechanical advantage around the quadrate-mandibular joint to make a major contribution to bite force.

There are several functional implications of this two-joint jaw suspension system. During ingestion of food, when wide gape and fast movements are primarily important, movement around the quadrate-mandibular joint allows wide excursion of the mandible. However, when the animal bites against resistance, muscles act around both the quadrate-mandibular and quadrate-squamosal joints. The position of the pterygoideus muscle is such that it may produce fast closing around the quadrate-mandibular joint during ingestion and powerful adduction around the quadrate-squamosal joint during hard biting. With this two-joint system, the pterygoideus muscle avoids potential mechanical compromises between power and speed. Further, lizards with large pterygoideus muscles develop an effective force-producing muscle without interfering with gape. Increasing the size of the pseudotemporalis muscle would, for example, increase potential bite force, but the position of this muscle is such that gape would be severely restricted.

This scheme applies generally to all living lizards because they all have streptostylic quadrates, but is must be carefully applied to fossil reptiles. The rhyncocephalian, *Glevosaurus*, and the prolacertilians[12] possess an interrupted lower temporal arcade, but fused quadrate-pterygoid and quadrate-squamosal junctions. In neither of these forms is the quadrate free swinging or streptostylic. Only in the true Squamata, including early forms such as the Paliguanidae[14], *Kuehneosaurus*[12] and *Icarosaurus*[13] was the streptostylic condition achieved. In the lepidosaurian reptiles the lower temporal arcade has been lost at least three times independently; however, in many forms this loss is not related to quadrate mobility. As well as lizards, snakes and birds also have streptostylic quadrates, but their skull morphology is such that in morphological and functional detail there is little real equivalence in the three systems, and the scheme applied here does not apply.

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Selective reconstitution of nude mice with long-term cultured and cloned specific helper T cells

R. Tees & M. H. Schreier

Basel Institute for Immunology, Grenzacherstrasse 487, Postfach, 4005-Basel, Switzerland

An antibody response is the end result of complex interactions among T cells, adherent cells and B cells[1-3]. An understanding of the interactions involved has proved difficult as pure populations of these cells have not been available. By making use of T-cell growth factor[4,5], we were able to grow normal helper T cells specific for heterologous erythrocytes[6]. Because specificity and mechanism of action of these cells had been demonstrated solely in culture, we sought to establish their competence in the whole animal. We have therefore examined here whether antigen-specific helper T cells, maintained in culture over long periods, would enable syngeneic nude mice to respond to T-cell-dependent antigens. The results show that specific helper T cells, propagated in serum-free medium *in vitro* for up to 15 months, can selectively and specifically reconstitute syngeneic C57BL/6J *nu/nu* mice. Depending on the specificity of the injected helper T cells, such nude mice could respond to sheep red blood cells (SRC) but not to horse red blood cells (HRC) and vice versa. The magnitude of the response was comparable to that of normal mice and could exceed it by almost 10-fold, depending on the source and number of injected helper T cells.

Table 1 documents the *in vitro* helper activity of the long-term cultured, cloned and subcloned helper T cells at the time of the present report. 10^3 to $10^{3.5}$ of these cells provided maximal T-cell help if added to 2×10^5 nude spleen cells in the presence of homologous antigen. A higher input of helper T cells rendered the culture medium acid and lowered the numbers of IgM plaque-forming cells (PFC). No PFC were induced in response to the unrelated antigen, indicating that these T cells were specific and that no polyclonal activation had occurred by day 5 of culture.

Injection of uncloned helper T cells (cultured over 3–4 months) with homologous antigen into syngeneic nude mice, induced a specific antibody response in these animals (Table 2). 5×10^6 HRC-specific uncloned helper T cells (LTC 15) gave rise to about five times as many direct PFC as could be expected in a primary response of normal mice 5 days after immunisation[7]. The simultaneous injection of the uncloned SRC-specific helper T-cell line LTC 25 and antigen (Table 2) gave rise to almost 10^6 direct PFC on day 5. The response to the unrelated antigen hardly exceeded background even when both antigens were injected at the same time (LTC 3, Table 2).

The unusually high response may reflect the lack of regulatory mechanisms in the nude mouse, such as 7S feedback inhibition[8] or the absence of suppressor T cells[9]. This is also indicated by the kinetics of the response. In contrast to normal mice, there was no sharp decrease in the number of PFC beyond day 5 after immunisation. Most reconstituted mice showed high or even increasing numbers of PFC up to day 7 after immunisation. Beyond that, the number of PFC varied considerably in individual mice before returning to background around 10 days after inoculation.

We have not determined what fraction of the injected helper T cells homes to the sites of antibody formation in the spleen. This aspect has an even stronger bearing on our reconstitution experiments with clones of helper T cells kept in continuous culture for up to 15 months. Table 2 shows results obtained with two of our clones and with one subclone (18/33/3). Although the number of PFC is somewhat lower than that obtained with uncloned helper T cells, it is still entirely comparable with the response of a normal mouse. The high degree of specificity remains unchanged. The antigen-independent anti-HRC response of subclone 18/33/3 (Table 2, last line) is under investigation.

The fact that a single clone of helper T cells can reconstitute a primary antibody response of normal magnitude raises the question of whether assumed restrictions in T-cell–B-cell interaction, operating at the level of idiotypes[10] or on a subset of cells[11], are compatible with our findings. It should be remembered that a nude spleen (on average 8×10^7 cells) contains at least 20,000 B-cell precursors, which can recognise the antigen SRC and are susceptible to T-cell help[12]. This minimal estimate does not account for antigen-specific recruitment of lymphocytes[13]. As 10^3 PFC can arise *in vitro* from one precursor within 5 days[12], it is obvious that the induction of less than 1% of B-cell precursors would be sufficient to give rise to about 10^5 PFC by day 5 after immunisation. Idiotypic analysis of the antibodies in normal and clonally reconstituted mice may provide critical information. Our preliminary results suggest that significant differences at the antibody level may exist between such mice. Thus, although the serum of the clonally reconstituted mice (18/33/3, Table 2) gave a haemagglutination end point when diluted 1 in 28,000, the average titres were significantly lower in mice reconstituted with uncloned specific helper T cells, even

Table 1 *In vitro* helper activity of long-term cultured, cloned or subcloned specific helper T cells at the time of *in vivo* reconstitution experiments

No. of T cells added per culture	Designation of T-cell line (LTC), clone or subclone (specificity given in parentheses)											
	LTC 15 (T'_{HRC})		LTC 3 (T'_{SRC})		LTC 25 (T'_{SRC})		Clone 18/19 (T'_{HRC})		Clone 1/25 (T'_{SRC})		Subclone 18/33/3 (T'_{HRC})	
	Antigen present in the culture (no. of PFC per culture directed against this antigen)											
	HRC	SRC	HRC	SRC	HRC	SRC	HRC	SRC	HRC	SRC	HRC	SRC
10^4	ND		ND		0	10,973 ± 11,422	ND		ND		2,470 ± 1,543	0
$10^{3.5}$	3,900 ± 2,561	0	0	6,540 ± 2,907	0	11,440 ± 5,312	7,260 ± 3,710	0	0	2,520 ± 3,710	9,813 ± 1,360	0
10^3	6,440 ± 2,850	0	0	9,410 ± 5,849	0	3,620 ± 825	8,740 ± 3,355	0	0	3,910 ± 1,748	2,630 ± 959	0
$10^{2.5}$	2,195 ± 1,440	0	0	1,455 ± 681		ND	890 ± 382	0	0	1,110 ± 167	ND	
10^2	40 ± 14	0	0	25 ± 21		ND			0	137 ± 145	ND	

Helper T cells were tested for helper activity and specificity in a specific *in vitro* helper assay[12]. 2×10^5 spleen cells from C57BL/6J *nu/nu* mice were cultured in a volume of 0.2 ml in flat bottom Falcon II tissue culture plates in the presence of 5×10^5 SRC or HRC and the indicated number of helper T cells. The culture medium was IMDM[15], supplemented with bovine serum albumin (1 mg ml^{-1}), human transferrin (30 μg ml^{-1}), soybean lipids (100 μg ml^{-1}) and cholesterol (50 μg ml^{-1})[12,15,16]. PFC were enumerated after 5 days in a modified Jerne plaque assay[17]. They are expressed as direct PFC per culture ± standard deviation of the mean. The helper T cells, specific for SRC or HRC (T'_{SRC}, T'_{HRC}) were obtained from spleens of lethally irradiated mice which had been reconstituted 7 days previously with thymocytes and injected with SRC or HRC (*in vivo* activated helper T cells (refs 12, 18)). They were cultured in the presence of antigen in Falcon 3013 tissue culture flasks in the same medium as described for the *in vitro* helper assay at a concentration of 10^6 activated T cells per ml. Antigen and irradiated syngeneic peritoneal cells (3,300R) were replenished at 1–2-week intervals. After 2–3 months of culture vigorous growth of cells was induced by supplementing the medium with partially purified T-cell growth factor[6]. The T-cell lines LTC15, 3 and 25 were subcultured and grown for another 3–4 months in TCGF-supplemented medium in the absence of antigen and peritoneal cells. Such T-cell lines were cloned (clone 18/19 and 1/25) and subcloned (18/33/3) in conditions of limiting dilution with a cloning efficiency of 5–20% (ref. 6). None of the T cells was exposed to antigen 3–4 months before the experiment.

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Table 2 Specific reconstitution of C57BL/6J *nu/nu* mice with long-term cultured or cloned helper T cells specific for HRC (T'_{HRC}) or SRC(T'_{SRC})

T cells (line, clone or subclone)	No. of T cells injected per mouse	No. of mice per group	Antigen injected	PFC per spleen directed against HRC	SRC
LTC 15 (T'_{HRC})	Nil	5	HRC	1,130 ± 277	304 + 210
	10^5	4	HRC	2,350 ± 290	ND
	10^6	5	HRC	53,680 ± 11,356	ND
	5×10^6	4	HRC	580,000 ± 76,302	272 ± 166
LTC 3 (T'_{SRC})	4×10^6	2	SRC + HRC	575 ± 412	268,450 ± 26,474
LTC 25 (T'_{SRC})	5×10^6	4	SRC	622 ± 398	940,250 ± 52,143
	5×10^6	4	HRC	1,746 ± 1,308	1,345 ± 1,163
18/19 Clone (T'_{HRC})	2×10^6	2	HRC	52,727 ± 14,978	274 ± 134
1/25 Clone (T'_{SRC})	5×10^6	4	SRC	485 ± 95	64,377 ± 4,919
18/33/3 Subclone (T'_{HRC})	5×10^6	4	HRC	159,100 ± 11,172	460 ± 254
	5×10^6	4	SRC	14,380 ± 1,951	965 ± 306

C57B1/6J *nu/nu* mice, 9th or 10th backcross (Institute for Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland) were injected intravenously with long-term cultured or cloned helper T cells, together with 5×10^6 SRC and/or HRC. Five days later the number of direct PFC in the spleen, directed against either antigen, was determined in a modified Jerne plaque assay[17].

though the number of PFC per spleen was considerably higher (for example, LTC 25, Table 2).

At present, we know little about the long-term fate of these injected helper T cells *in vivo*. Boosting of mice which had been reconstituted with the SRC-specific clone 1/25 and primed 21 days earlier, gave rise to even higher numbers of PFC against the homologous antigen. This suggests that these helper T cells survive in the recipient animal. As nude mice cannot generate T-cell growth factor[19], an absolute requirement for T-cell proliferation[4,5], the reconstituting cells may revert there to a resting state. In the experiments presented here, we studied a few clones only, and have not observed a significant switch to IgG production. By using various clones and immunisation schedules, we should be able to decide whether the same helper T cell can provide help for various antibody classes.

Our results show that helper T cells reveal the same degree of specificity *in vitro* and *in vivo*. *In vitro*, these helper T cells are H-2 restricted, the restriction mapping to the left of the I-B region[14]. Reconstitution of T-cell-deficient mice with T cells selected and passaged in culture provides a new tool for studying the function and regulation of specific helper T cells, and should prove especially useful in discriminating between useful and misleading *in vitro* phenomena.

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Vitamin B_{12} synthesis by human small intestinal bacteria

M. J. Albert, V. I. Mathan & S. J. Baker*

Wellcome Research Unit, Christian Medical College Hospital,
Vellore-632004, Tamil Nadu, India

In man, physiological amounts of vitamin B_{12} (cyanocobalamin) are absorbed by the intrinsic factor mediated mechanism exclusively in the ileum[1]. Human faeces contain appreciable quantities of vitamin B_{12} or vitamin B_{12}-like material presumably produced by bacteria in the colon[2], but this is unavailable to the non-coprophagic individual. However, the human small intestine also often harbours a considerable microflora[3–6] and this is even more extensive in apparently healthy southern Indian subjects[6]. We now show that at least two groups of organisms in the small bowel, *Pseudomonas* and *Klebsiella* sp., may synthesise significant amounts of the vitamin.

Bacteria were obtained by aspiration from the jejunum and ileum of healthy southern Indian control subjects, and patients with tropical sprue. All subjects gave their informed consent to the study and all had normal vitamin B_{12} absorption. Full details of the intubation, bacteriological techniques and vitamin B_{12} absorption tests have been described elsewhere[6]. Representative isolates of different groups of aerobic and anaerobic organisms were grown in the medium described by Nishio *et al.*[7] modified to support the growth of fastiduous organisms[8]. Vitamin B_{12} activity was measured in the presence of potassium cyanide by microbiological assay using *Euglena gracilis* Z strain[9] and *Ochromonas malhamensis*[10] as the test organisms. Descending paper chromatography was carried out in *sec*-butanol/acetic acid/water[11] and bioautography by the method of Linnel *et al.*[12].

Using the *Euglena gracilis* assay, the following groups of organisms were found to produce no detectable vitamin B_{12}-like material (the numbers in parentheses indicate the number of strains tested): enterobacteria (10), *Escherichia coli* (17), streptococci (18), *Neisseria* (5), staphylococci (4), aerobic lactobacilli (12), bifidobacteria (11), and *Bacteroides* (8). With the same assay, five groups of organisms produced considerable amounts of vitamin B_{12}-like material (Table 1). A subsample of these five groups of organisms were also tested using the *Ochromonas* assay (Table 1). Since the growth response of *Ochromonas* is more specific for vitamin B_{12} than that of *Euglena*[10], the material produced by the clostridia, *Veillonella* and *Fusobacteria* presumably consists of vitamin B_{12} analogues, whereas that produced by *Pseudomonas* and *Klebsiella* probably is biologically active vitamin B_{12}. This was further supported by chromatography and bioautography of the material produced by *Pseudomonas* which gave an R_f value identical to that of cyanocobalamin. A comparison of the whole broth and bacteria-free filtrates showed that in the case of *Klebsiella* the vitamin B_{12} activity was associated with the bacterial bodies but that with *Pseudomonas* the activity was principally free in the supernatant.

It has been shown that free intrinsic factor is often present in the lumen of the small intestine[13]. If these organisms also produce vitamin B_{12} *in vivo*, the free vitamin B_{12}, or that bound to bacteria, after undergoing liberation by digestive enzymes, could bind with the free intrinsic factor and, if absorbed, contri-

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* Present address: Department of Medicine, University of Manitoba, 409 Tache Avenue, Winnipeg, Manitoba R2H 2A6, Canada.

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Table 1 Vitamin B_{12} activity of bacteria isolated from jejunal aspirates

	Euglena gracilis assay			*Ochromonas malhamensis* assay		
	No. of strains tested	Vitamin B_{12}-like activity ($\mu g\ l^{-1}$ culture)		No. of strains tested	Vitamin B_{12}-like activity ($\mu g\ l^{-1}$ in culture)	
		Mean	Range		Mean	Range
Pseudomonas	7	15.0	13.0–17.3	3	14.0	10.8–18.8
Klebsiella	10	2.9	1.5–7.2	2	3.2	1.4–5.0
Lecithinase-positive clostridia	7	14.0	5.2–37.9	2	0.1	0–0.2
Veillonella	13	14.3	7.2–40.0	3	0.6	0–0.9
Fusobacteria	6	26.6	9.2–36.0	3	0	0–0

bute significantly to the vitamin B_{12} intake of the individual. For example, 10 ml of a culture of *Pseudomonas* would provide 0.14 μg of the vitamin which is more than the minimum daily dose of 0.1 μg which will produce a haematological response in subjects with vitamin B_{12} deficiency megaloblastic anaemia[14].

The vitamin B_{12} analogues produced by the bacterial isolates were not identified further. Those with no alteration in the corrin ring or benzimadazole moeity can also combine with free intrinsic factor[15,16]. However, since the amount of intrinsic factor passing through the intestine is in considerable excess of that needed to absorb the minimum daily vitamin B_{12} requirements[13], the appropriate analogues would have to be present in considerable amounts significantly to reduce the amount of intestinally produced vitamin B_{12} absorbed by normal subjects.

The role of intraluminal small intestinal bacteria in human vitamin B_{12} metabolism appears complex. Subjects with small intestinal bacterial overgrowth associated with a stagnant bowel syndrome, frequently have vitamin B_{12} malabsorption[17,18]. This malabsorption may often be reversed by the administration of broad spectrum antibiotics[18], suggesting that the defect is closely related to the bacterial overgrowth. However, the precise mechanism for the vitamin B_{12} malabsorption in these subjects is not clear. Various mechanisms have been suggested, including the bacterial uptake of vitamin B_{12} (ref. 19) or the intrinsic factor B_{12} complex[20,21]; alteration of the IF–B_{12} complex[22]; the production of toxins[23] which may affect the binding of the IF–B_{12} complex to the receptor[24]; and the production of large amounts of vitamin B_{12} analogues[25] blocking vitamin B_{12} absorption. In apparently healthy southern Indian subjects with normal vitamin B_{12} absorption there is frequently considerable bacterial colonisation of the upper small intestine[6], but the degree of bacterial overgrowth is not as great as in patients with a stagnant bowel syndrome with vitamin B_{12} malabsorption[26]. This fact, and the finding in the present study that some organisms produce predominantly vitamin B_{12}-like material (*Ochromonas malhamensis* growth factor) while others produce only vitamin B_{12} analogues (*Euglena gracilis* but not *Ochromonas malhamensis* growth factor), suggests that the quantity and type of the luminal flora may be important in determining the role of the intestinal bacteria in the vitamin B_{12} ecology of the individual.

In the light of the findings reported here, it is of interest that vitamin B_{12} deficiency among vegetarian Indians has been found with relatively high frequency in Indian immigrants in England[27–30], whereas it is uncommon among the same people in India with identical dietary patterns[31]. It is possible that in India these individuals have a bacterial flora in the small intestine which provides a significant proportion of their daily requirements of vitamin B_{12}, but when they move to a more protected environment the bacterial flora reverts to that characteristic of subjects living in western countries with little or no resident flora in the upper small bowel[3], the individuals lose their endogenous source of vitamin B_{12} and therefore become more prone to develop overt vitamin B_{12} deficiency.

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A factor(s) in *Klebsiella* culture filtrates specifically modifies an HLA-B27-associated cell-surface component

A. F. Geczy, Kerri Alexander & Helen V. Bashir

NSW Red Cross Blood Transfusion Service, 153 Clarence Street, Sydney, NSW, 2000, Australia

J. Edmonds

Rheumatology Department, The St George Hospital, Kogarah, NSW, 2217, Australia

We have previously shown[1,2] that a serum raised against certain isolates of *Klebsiella pneumoniae* lyses the lymphocytes of HLA-B27-positive patients with ankylosing spondylitis (AS) but not of B27-positive or B27-negative healthy controls. These observations suggested that some *Klebsiella* antigens cross-react with a gene product intimately associated with B27 or possibly with 'modified' B27 (refs 1, 3) in patients with AS. It seems likely therefore, that some *Klebsiella* antigens play a role in the pathogenesis of AS, perhaps by specifically modifying a B27-associated cell-surface marker. We have now examined the influence of culture filtrates from three *Klebsiella* isolates on lymphocytes from B27-positive and B27-negative healthy individuals. We report that 24-h culture filtrates of *Klebsiella* K43 (previously *Klebsiella* F19) contain a factor(s) capable of specifically modifying the sensitivity to anti-*Klebsiella* K43 serum of $B27^+AS^-$ lymphocytes (that is, cells not lysed by anti-*Klebsiella* K43 serum): 'modified' $B27^+AS^-$ lymphocytes are now serologically similar to the cells of B27-positive patients with AS ($B27^+AS^+$). The failure of anti-*Klebsiella* F10 and of anti-*Escherichia coli* sera to lyse lymphocytes, which have been incubated with the corresponding culture filtrates, excludes a nonspecific lysis by the anti-bacterial sera of target lymphocytes bearing bacterial antigens randomly distributed on their surface. Therefore, the data are compatible with a specific transformation by a *Klebsiella* K43-derived soluble factor of a B27-associated lymphoid cell component.

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Bacterial cultures were isolated from faecal and urine samples (*Klebsiella pneumoniae* K43, *Klebsiella* isolates F10, F12 and 462; *E. coli*) and from a wound swab (*Staphylococcus* sp.). Bacteria were grown in Tryptone soya broth (TSB, Oxoid) and killed by adding 0.25 ml of 40% formaldehyde to a 20 ml suspension of the organisms ($\sim 10^9$ per ml) in nutrient broth. The formalin-killed bacteria were washed twice with sterile 0.9% NaCl and stored at −20 °C. Antisera were raised in rabbits (3–5 kg) against formalin-killed suspensions of the bacteria as previously described[1]. Briefly, the animals were injected intravenously (i.v.) with about 10^9 bacteria in normal saline (2 ml) and 2 weeks later, intramuscularly with 10^{10} organisms in 2 ml complete Freund's adjuvant (Difco). Ten days later, the rabbits were boosted i.v. with 1 ml of bacterial suspension (10^8–10^9 organisms) in normal saline. Two weeks after the last injection, the animals were bled and the serum stored at −20 °C.

The results in Table 1 show that the lymphocytes of B27^{+}AS^{-} individuals, incubated for 24 h in TSB or with culture filtrates of *Klebsiella* F10, F12 or with *E. coli*, are not lysed by anti-*Klebsiella* K43 serum. Similarly, anti-*Klebsiella* K43 serum is not cytotoxic for the lymphocytes of B27^{-}AS^{-} or of B27^{-}AS^{+} individuals following a 24-h incubation with any of the bacterial culture filtrates. On the other hand, the strong reactivity to anti-*Klebsiella* K43 serum of B27^{+} cells from patients with ankylosing spondylitis (by Rome and New York criteria)[4] is preserved even when these cells are cultured for 24 h in the four different culture filtrates. By contrast, B27^{+}AS^{-} lymphocytes, incubated with culture supernatants of *Klebsiella* K43, become susceptible to lysis by anti-*Klebsiella* K43 serum but not by antisera to *Klebsiella* isolates F10, 462 or to *E. coli* and *Staphylococcus* sp. Furthermore, no 'extra' serologically-defined specificities are detected when *Klebsiella* K43-incubated B27^{+}AS^{-} cells were tested against a panel of antisera defining 13 HLA-A and 20 HLA-B specificities (results not shown). The transformation of B27^{+}AS^{-} lymphocytes appears to be a specific event and not a consequence of randomly-attached *Klebsiella* K43 determinants on the surface of B27^{+}AS^{-} cells. This conclusion is supported by the failure of anti-*Klebsiella* F10 serum to lyse lymphocytes incubated with culture filtrates of *Klebsiella* F10. As B27^{-}AS^{-} and B27^{-}AS^{+} lymphocytes are not

Table 1 Culture filtrates of *Klebsiella* K43* specifically modify a B27-associated cell surface component on the lymphocytes of B27-positive healthy individuals but not on the cells of B27-negative healthy controls; the lymphocytes of B27-negative patients with AS are also not modified

Incubation	Serum	Target lymphocytes B27^{+}AS^{-}	B27^{-}AS^{-}	B27^{-}AS^{+}	B27^{+}AS^{+}
Nutrient broth	Anti-B27	51	0	4	60
	Anti-*Klebsiella* K43	2	1	2	81
	Anti-*Klebsiella* F10	10	0	0	1
	Anti-*Klebsiella* 462	5	0	NT	6
	Anti-*E. coli*	4	0	18	5
	Anti-*Staphylococcus*	3	8	NT	NT
Culture filtrate of *Klebsiella* K43	Anti-B27	49	5	0	69
	Anti-*Klebsiella* K43	71	9	7	85
	Anti-*Klebsiella* K10	7	12	0	1
	Anti-*Klebsiella* 462	7	9	1	1
	Anti-*E. coli*	9	4	0	5
	Anti-*Staphylococcus*	4	6	NT	NT
Culture filtrate of *Klebsiella* F10	Anti-B27	58	4	7	71
	Anti-*Klebsiella* K43	11	4	7	71
	Anti-*Klebsiella* F10	3	4	7	0
	Anti-*Klebsiella* 462	10	4	NT	NT
	Anti-*E. coli*	9	5	4	0
	Anti-*Staphylococcus*	NT	0	NT	NT
Culture filtrate of *Klebsiella* F12	Anti-B27	65	2	1	52
	Anti-*Klebsiella* K43	9	7	7	63
	Anti-*Klebsiella* F10	0	3	2	0
	Anti-*Klebsiella* 462	3	2	1	1
	Anti-*E. coli*	7	3	0	7
	Anti-*Staphylococcus*	0	NT	NT	NT
Culture filtrate of *E. coli*	Anti-B27	60	2	16	91
	Anti-*Klebsiella* K43	10	5	14	85
	Anti-*Klebsiella* F10	12	4	7	6
	Anti-*Klebsiella* 462	6	7	16	16
	Anti-*E. coli*	12	9	11	13
	Anti-*Staphylococcus*	1	0	NT	NT

Peripheral blood lymphocytes were obtained from groups of B27-positive normal controls (B27^{+}AS^{-}, $n = 19$), B27-negative normal controls (B27^{-}AS^{-}, $n = 17$), B27-positive patients with ankylosing spondylitis (B27^{+}AS^{+}, $n = 6$) and from B27-negative patients with ankylosing spondylitis (B27^{-}AS^{+}, $n = 3$). The cells were separated from heparinised blood by centrifugation in Ficoll–Paque (Pharmacia) for 25 min at 580*g*. The leukocyte band was removed and washed with RPMI 1640 medium (Flow) containing 2 ml heparin (1,000 units ml^{-1}), 10 ml IM HEPES (Sigma) buffer, 5 ml L-glutamine (200 mM) and 2 ml penicillin-streptomycin (5,000 IU, 5,000 μg ml^{-1}; Flow) per 500 ml of RPMI medium. The leukocytes were suspended in RPMI medium containing 10% human AB serum and centrifuged for a further 10 min at 180*g*. The cells were then resuspended in RPMI medium containing 20% human AB serum and adjusted to a cell concentration of 2.5×10^6 mononuclear cells per ml of medium. The incubation mixture consisted of 3.8 ml of cell suspension in RPMI containing 20% human AB serum and either 0.2 ml of TSB (Oxoid; diluted 1 : 2 with Dulbecco phosphate buffer (DPB) just before use) or 0.2 ml of culture filtrate (diluted 1 : 2 with DPB). Higher concentrations of culture filtrate were non-specifically cytotoxic to human leukocytes. Both the TSB and the culture filtrates were Millipore-filtered before dilution with DPB. The lymphocytes were incubated with TSB or with the various culture filtrates at 37 °C for 24 h. They were then washed twice with RPMI containing 10% human AB serum and resuspended at a concentration of 3×10^6 cells per ml. About 6×10^6 lymphocytes, suspended in 2 ml of RPMI containing 10% human AB serum, were labelled with 100 μCi of ^{51}Cr (0.1 ml; 200–500 μCi μg^{-1}, Amersham) for 60 min at 37 °C. The cells were then washed twice with RPMI + 10% AB serum and resuspended at a concentration of 3×10^6 cells per ml. One hundred μl of the ^{51}Cr-labelled cell suspension were added to 10 × 75-mm plastic tubes and incubated with 100 μl of antiserum at 24 °C for 30 min. Rabbit complement (100 μl) was then added to each tube and the incubation continued for a further 60 min. The cells were centrifuged at 2,000 r.p.m. for 15 min and 100 μl of the supernatant was counted in a Packard autogamma scintillation spectrometer. The amount of radioactivity in the samples was compared with radioactivity present in an equal volume of the supernatant from tubes containing cells in medium plus complement only, and with that present in tubes containing cells which had been lysed by the addition of 200 μl of Nonidet-P40. The results are expressed as the percentage of maximum ^{51}Cr released, which was calculated as follows:–

$$^{51}\text{Cr release } \% = 100 \times \frac{(\text{Radioactivity released by antiserum}) - (\text{Radioactivity released in absence of antiserum})}{(\text{Radioactivity released by NP-40-solubilised cells}) - (\text{Radioactivity released in absence of antiserum})}.$$

The results shown are of a representative experiment on the cells of single individuals for each category. All sera were tested undiluted. NT, not tested.

* *Klebsiella* K43 is a standard capsular designation; *Klebsiella* F10 and 462, antisera to which do not lyse B27^{+}AS^{+} lymphocytes, are two different isolates which have not yet been serotyped.

Table 2 Reactivity to anti-*Klebsiella* K43 serum of B27$^+$AS$^-$, B27$^-$AS$^-$ and B27$^-$AS$^+$ lymphocytes incubated with culture filtrates of *Klebsiella* K43

Diagnosis	B27-positive	No. transformed no. tested
Normal	Yes	17/19
Normal	No	0/17
Definite AS	No	0/3

Diagnosis was based on Rome and New York criteria. Cells are regarded as transformed if at least 50% are lysed by anti-*Klebsiella* K43 serum after incubation with K43 filtrate.

transformable by any of the *Klebsiella* culture filtrates tested, it appears that B27 or the product of a closely-linked gene is a specific target for a *Klebsiella* K43-derived factor(s).

Table 2 summarises the data on the reactivity to anti-*Klebsiella* K43 serum of B27$^+$AS$^-$, B27$^-$AS$^-$ and B27$^-$AS$^+$ lymphocytes incubated with culture filtrates of *Klebsiella* K43. The results indicate that none of the cells of B27-negative patients with AS nor of B27-negative normal controls is transformable by the *Klebsiella* K43-derived culture supernatant. However, two of the nineteen B27$^+$AS$^-$ cells have failed to be 'converted' by the *Klebsiella* K43 culture filtrate. It is apparent (Table 1 and Table 2) that only some strains of *Klebsiella* can trigger the specific transformation of B27$^+$AS$^-$ cells and it is possible that some individuals carry a 'subtype' of B27 which lacks a hypothetical receptor required to specifically bind a *Klebsiella* K43 antigenic determinant. This effect has also been demonstrated with another serotype of *Klebsiella* (K21; serotyped by Dr. I. Ørskov), indicating that the cross-reactivity of B27$^+$AS$^+$ cells with some strains of *Klebsiella* is not related to the capsular serotype of the organsim.

Although the chemical nature and mechanism of action of *Klebsiella*-derived factors are not yet understood, preliminary results indicate that they are non-dialysable but heat labile at 56 °C for 30 min. (results not shown).

Bacterial products have generally been shown to have a nonspecific influence on the immune response. Thus, culture filtrates and cell wall-rich fractions of *Listeria monocytogenes* contain substances that are mitogenic for B lymphocytes *in vitro*[5,6] while *in vivo* they, (1) function as immunologic adjuvants[7,8], (2) activate macrophages[5] and (3) induce a transitory state of nonspecific resistance to infection by live *Listeria*[5,9].

Furthermore, lipopolysaccharide, dextran sulphate, mycobacteria[10] and peptidoglycan[11] have been shown to be B cell-specific polyclonal activators of mouse lymphocytes. To our knowledge, the studies reported here represent the first demonstration that bacterial antigens may have a specific effect on HLA-associated gene products. It is tempting to suggest that the modification by environmental agents of specific cell surface components is an important step in the pathogenesis of the HLA-linked seronegative arthropathies.

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Covalent attachment of a progestational steroid to chick oviduct progesterone receptor by photoaffinity labelling

Leon S. Dure IV, William T. Schrader & Bert W. O'Malley

Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030

The chick oviduct progesterone receptor has been characterised and purified by us[1-3] and shown to consist of a mixture of two hormone-binding proteins[4,5]. These two proteins have equivalent ligand-binding sites, to which progesterone and other progestational steroids bind with high affinity ($k_{diss} = 10^{-9}$ M)[6,7]. Due to the noncovalent nature of this interaction, analysis of the proteins in denaturing or nonequilibrium conditions has always been hampered by ligand–protein dissociation during the experiments ($t_{1/2}$ = 12 h at 0 °C, 25 min at 37 °C)[6]. Efforts to use covalent attachment of steroids by alkylation or photoactivation have been largely unsuccessful, both for this protein and for other receptors[8]. However, covalent labelling of steroid isomerases[9] and an androgen-binding protein[10] has been obtained in reasonable yields using photoactivation of keto steroids containing multiple double bonds in conjugation with the ring-A ketone. We now report the first successful covalent attachment of a radiolabelled steroid to its receptor protein. The ligand chosen was a synthetic progestin, 17α,21-dimethyl-19-nor-pregn-4,9-diene-3,20-dione (R5020; Roussel-Uclaf)[11]. R5020 is available as the tritiated 17α-methyl derivative ($\geq$50 Ci mmol^{-1}) and has been used extensively for measuring mammalian progesterone receptors in normal[12,13] and neoplastic tissues[14,15]. This steroid was chosen for the photoaffinity study because its absorption maximum is at 320 nm, thereby allowing its activation by UV light at wavelengths above 300 nm, where protein damage would be minimised. The method should be generally applicable to other progestin receptors.

Oviduct cytosol from immature, oestrogenised chicks was prepared in buffer A (10 mM Tris-HCl, *p*H 7.4, 1 mM Na$_2$EDTA, 12 mM (1-thioglycerol) at 4 ml buffer per g tissue, as described previously[16]. The progesterone receptors were labelled with ^{3}H-R5020 at 2×10^{-8} M for 2 h at 0 °C. Then the hormone–receptor complexes were precipitated at 30% saturation of ammonium sulphate in buffer A and redissolved in a volume of buffer A equal to the original cytosol volume. Equilibrium binding studies showed R5020 binding to the progesterone receptor, as determined by (1) competition and displacement by authentic progesterone, (2) labelling of the same number of binding sites in the preparation by either labelled R5020 or progesterone and (3) chromatographic analysis of R5020–receptor complexes by DEAE-cellulose, gel filtration and sucrose-gradient ultracentrifugation. In all these tests, R5020–receptor complexes were indistinguishable from the complexes formed by authentic progesterone. Finally, binding of ^{3}H-R5020 to nonreceptor proteins (nonspecific weak absorption) was less than 5%.

Redissolved complexes were then photolysed at 0 °C in an ice bath using an 85-W low-pressure mercury vapour lamp (General Electric H85A3). Samples of receptor were placed in a beaker covered by a borosilicate glass plate to absorb UV below 300 nm. Aliquots (1.0 ml) were removed at intervals for up to 2 h.

Samples were tested initially for covalent steroid coupling by (1) addition of an equal volume of ice-cold 10% (w/v) of trichloroacetic acid (TCA). Precipitated protein was redissolved and counted for ^{3}H. There was a time-dependent increase in precipitable ^{3}H during UV illumination. Covalency was further

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assessed by (2) vigorous, repeated (five times) extraction with a 20-fold excess of diethyl ether, (3) gel filtration of irradiated samples in 5.0 M urea and (4) dialysis of the complexes against 1% (w/v) SDS. In all four types of test, UV-ray irradiation for 60 min resulted in optimal coupling of ^{3}H-R5020 to acid-insoluble material with a nominal coupling efficiency of 10–20% of the input ligand. Thus, the photoactivation reaction was indeed resulting in covalent attachment of the steroid to protein. We used electrophoresis in denaturing conditions by the method of Laemmli[2,3,17] to determine whether ^{3}H-R5020 was, in fact, attached to the desired receptors.

The results of these experiments are shown in Fig. 1. In *a*, three equivalent cytosol preparations (1.0 ml each) were complexed with ^{3}H-R5020, ^{3}H-R5020 plus a 100-fold molar excess nonradioactive progesterone, or ^{3}H-progesterone alone. Saturation studies not shown here indicated that this amount of ^{3}H-R5020 was sufficient to occupy about 20% of the available receptor sites. At this ratio, negligible binding of ligand to non-receptor protein is detectable by competition assays. Figure 1*a* shows that the ^{3}H-R5020 was coupled to two major species, as indicated by the arrows. This labelling was eliminated by the presence of nonradioactive progesterone, whereas general labelling of the rest of the gel was not inhibited by the competitor. Labelled progesterone did not couple significantly to proteins, as expected, at a wavelength of >300 nm, which is the cutoff wavelength of the borosilicate glass filter. Figure 1*a* also shows considerable radioactivity running near the dye front; this material runs several fractions ahead of the front and probably represents ^{3}H-R5020 derivatives bearing charged groups produced by photolysis.

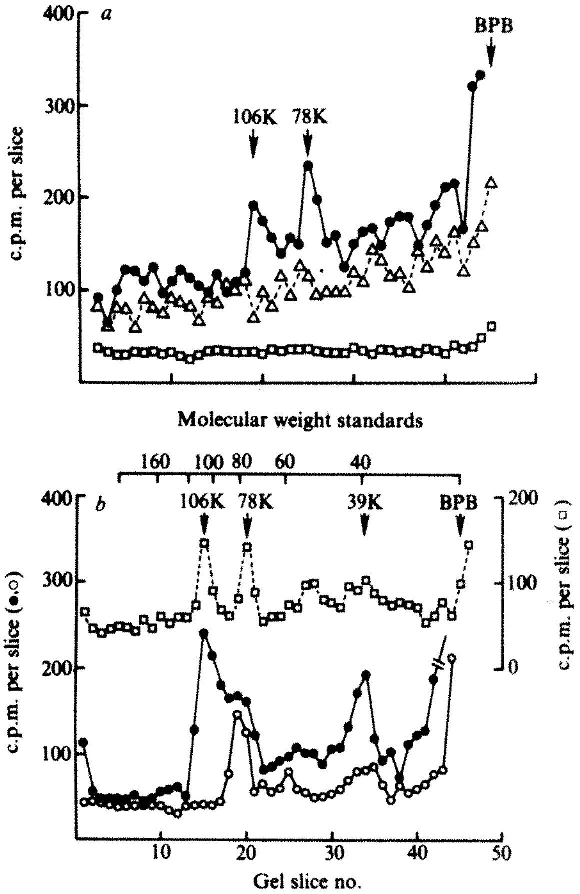

Fig. 1 Polyacrylamide gel electrophoresis of photolysed progesterone receptor preparations in denaturing conditions in 1% SDS by the method of Laemmli[17]. Total acrylamide monomer content was 7.5% in the separation gel. Gels were run in borosilicate glass tubes 6 mm × 10 cm, with 0.5-cm stacking gels. Electrophoresis of samples (10–100 µl containing 0.01% bromphenol blue tracking dye) was at 2.5 mA per gel until the dye front had travelled 10 cm. Gels were removed, sliced and minced using a Savant automated slicer and then counted for ^{3}H in Amersham-Searle ACS scintillation cocktail at 25% efficiency. *a*, Electrophoresis of three companion receptor samples after labelling, precipitation and UV-ray irradiation for 30 min as described in the text. Samples were labelled with ^{3}H-R5020 (●), ^{3}H-R5020 plus a 100-fold molar excess of nonradioactive progesterone (△), or with ^{3}H-progesterone (□). Arrows identify two major labelled species whose labelling was time and irradiation dependent. *b*, Molecular weight determination of the major photoaffinity-labelled ^{3}H-R5020–protein complexes. A standard MW curve was determined on companion gels as shown by the upper abscissa. Standards were 5 µg each of *Escherichia coli* RNA polymerase (165, 155, 95 and 39K bands), chicken conalbumin (86K), bovine serum albumin (68K) and chymotrypsinogen (26K). Protein bands were visualised by staining with Coomassie brilliant blue. Photolysed receptor–^{3}H-R5020 complexes were prepared by 60 min UV-ray irradiation followed by dialysis against buffer A and lyophilisation. The samples were then redissolved in 20% of the orginal volume of sample buffer containing 1% SDS, and subjected to electrophoresis as in *a*. Unfractionated receptors (□) were run as above or aliquots were chromatographed by stepwise KCl elution from DEAE-cellulose as described in the text to resolve receptor A and B proteins before electrophoresis. Two KCl steps were used: 0.15 M to yield protein A (○), and 0.3 M KCl to yield protein B (●).

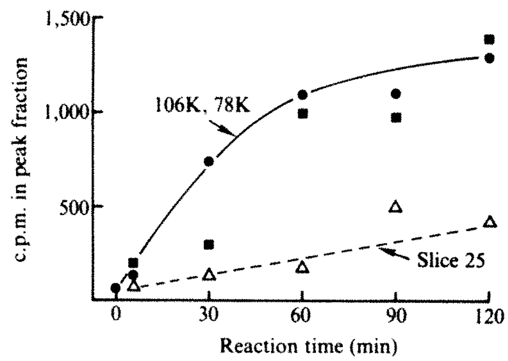

Fig. 2 Time course of photoaffinity labelling of progesterone receptors by ^{3}H-R5020. Chick oviduct cytosol was labelled for 120 min with 2×10^{-8} M ^{3}H-R5020 and then precipitated at 30% saturation of ammonium sulphate. The redissolved precipitate in buffer A was irradiated for the times indicated on the abscissa and then aliquots (1.0 ml) were frozen, lyophilised and subjected to SDS-polyacrylamide gel electrophoresis as described in Fig. 1 legend. The radioactivity in gel slices 13 (●), 18(■) and 25(△) are shown. These were the peak fractions corresponding to the labelled proteins of MWs 106,000, 78,000 and 39,000 respectively.

The extent of labelling seen on the gels was low, but consistent with the input noncovalent binding of ^{3}H-R5020 to receptor. For 150,000 c.p.m. ml^{-1} bound (by saturation analysis) and 10% efficient photoaffinity labelling (by TCA precipitation), the radioactivity which was recovered on the gels was about 50% of the theoretical value. This is a reasonably acceptable level when associated losses during transfer, dialysis and electrophoresis are considered.

We had shown previously[18] that the two progesterone-binding receptor proteins B and A had molecular weights (MWs) of about 115,000 and 79,000. Thus we determined the MWs of the two covalent ^{3}H-R5020 peaks by comparison with electrophoresis of protein standards. The results are shown in Fig. 1*b*. Again, two distinct radioactive peaks were seen with equivalent radioactivity in each. Their MWs were 106,000 and 78,000 in this experiment. A protein band in the preparation, migrating at 39,000 g mol^{-1} was also labelled significantly, as shown by the arrow at 39K. These results provided additional evidence that the labelled proteins were, in fact, the desired receptor subunits.

As a third test, a photolysed ^{3}H-R5020–receptor preparation was made and fractionated by DEAE-cellulose chromatography on a 3.0-ml column equilibrated in buffer A as described previously[4]. Stepwise KCl elution at 0.15 M specifically elutes receptor A purified about 80-fold over cytosol; subsequent elution at 0.3 M KCl elutes receptor B, purified about 200-fold over cytosol but contaminated with about 30% receptor A. Samples from 5-ml reactions were chromatographed, desalted by dialysis in buffer A and subjected to SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1*b*, 0.15 M KCl elution of ^{3}H-labelled receptor A yielded the 78,000 g mol^{-1} peak on the SDS gel; 0.3 M KCl elution yielded a major species at

106,000 g mol^{-1}. In addition, a shoulder occurred at 78,000 together with an augmented peak at 39,000. Thus, the DEAE step had resolved the two higher MW protein bands as predicted from the chromatographic behaviour of receptors A and B proteins.

To test the saturability and extent of the photoaffinity reaction to these proteins, a time course study of UV-ray irradiation was carried out using an excess of ^{3}H-R5020 as the label, and aliquots (1.0 ml) were removed at various times. The samples were run individually on SDS-polyacrylamide gels by the method in Fig. 1; the peak fraction radioactivity of MW 106,000 and 78,000 were then plotted as a function of irradiation time. The results are shown in Fig. 2. The experiment shows a time-dependent covalent attachment of the label to the two receptor peaks, with a plateau at 1–2 h. Radioactivity in the peaks was now over 1,000 c.p.m., consistent with the use of additional input ligand. Significantly, both peaks became labelled at equivalent rates.

At least one additional peak of radioactive protein had been seen on the gels in Fig. 1, having a MW of about 39,000. It was possible that this was an additional receptor form, perhaps a proteolytic degradation product produced by an endogenous protease[19]. A hormone-binding intermediate in the digestion of the receptors has an apparent native MW of about 43,000 (ref. 20) Alternatively, the 39,000 g mol^{-1} peak could be simply a non-receptor protein which became labelled due to its weak affinity for the ^{3}H-R5020. Three types of test have been done which indicate that the latter alternative is the correct explanation. First, as shown in Fig. 1, radioactivity bound to protein on the gel in the low MW region was not competitively inhibited by simultaneous addition of nonradioactive progesterone. Thus, this 39,000 band was not a pre-existing receptor form present in the extracts. Second, in data not presented here, gels stained for protein with Coomassie brilliant blue showed a prominent band of protein at 39,000 g mol^{-1}, whereas the two higher MW bands did not coincide with stainable protein bands in these crude preparations. Thus, a potential contaminating protein of the proper mobility exists in the extracts. Third, Fig. 2 shows that the labelling kinetics of this 39,000 band differed markedly from that of the two receptor peaks. We therefore consider that the 39,000 band is probably a major protein contaminant, and becomes nonspecifically labelled with ^{3}H-R5020.

We conclude that the photolytic method adopted here covalently attaches steroid R5020 to both progesterone receptor proteins in yields of about 10%. Additional side reactions also occur, resulting in at least labelling of a 39,000 g mol^{-1} protein which is not receptor. This side reaction may arise as a consequence of photoactivation of the steroid; in the activated π^* state the ligand may bind less well to the receptor binding site and thus be released to interact with other proteins in the solution. As the 39,000 band protein is present in vast excess over the receptors, the efficiency of receptor–adduct formation in the system is very great compared with side reactions.

These studies thus provide direct physical determination of the MWs of the progesterone receptor components and provide additional evidence for the existence of two dissimilar hormone-binding subunits. As the ligand attachment is covalent, these labelled complexes should be ideal substrates for probing the hormone-binding site and as reagents in receptor radioimmunoassay. The receptor–labelling method should be generally applicable to other progestin receptors, including, for example, those of interest in breast tumours. Irradiation of tumour cells containing ^{3}H-R5020 in tissue culture may also be a practical method for studying the ultimate fate of nuclear receptor–hormone complexes.

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Complete nucleotide sequence of immunoglobulin γ2b chain gene cloned from newborn mouse DNA

Yuriko Yamawaki-Kataoka, Tohru Kataoka, Naoki Takahashi, Masahiro Ôbata & Tasuku Honjo*

Department of Physiological Chemistry and Nutrition, Faculty of Medicine, University of Tokyo, Tokyo 113, Japan

Immunoglobulin heavy chain protein comprises five classes μ, γ, α, δ and ε chains which differ in amino acid sequences of the constant (C) region. The γ heavy chain class, which contains three domains and the hinge region, is divided into several subclasses such as γ1, γ2a, γ2b and γ3 in mouse. Amino acid sequence studies of mouse and human heavy chain proteins have shown that the γ subclass heavy chains are more closely related to each other than to the heavy chains of other classes. A similar conclusion was drawn from a nucleic acid hybridisation study using cDNAs complementary to the purified heavy chain mRNAs of the γ subclasses[1]. These results suggest that the immunoglobulin heavy chain genes of the γ subclasses have evolved by relatively recent gene duplication of the ancestral γ chain gene. The complete nucleotide sequence of the γ1 chain gene has demonstrated unequivocally that the domains and the hinge region are encoded in separate segments of DNA[2]. Partial nucleotide sequences of the γ2b chain gene indicated that the γ2b gene is interrupted by intervening sequences (IS) at locations homologous to those in the γ1 chain gene[3]. Comparison of the structures of these closely related genes would give new insights into evolution of eukaryotic genes and serve for understanding biological significance of various segments of the genes. We have now determined the complete nucleotide sequence of the immunoglobulin γ2b chain gene cloned from newborn mouse DNA. The sequence not only predicts the complete amino acid sequence of the γ2b chain but also demonstrates that the γ2b chain gene is interrupted by three intervening sequences at the junction of the domains and the hinge region.

The 6.6-kilobase *Eco*RI fragment coding for the C region of the immunoglobulin γ2b chain was previously cloned from newborn mouse DNA using λgtWES·λB as an EK2 vector[3]. The recombinant phage was referred to as λgtWES·IgH22 (abbreviated IgH22). The 6.6-kilobase fragment was isolated by agarose gel electrophoresis and ligated with the *Eco*RI-digested pBR322[4]. The hybrid plasmid was referred to as pBR322·IgH22 (abbreviated pIgH22). The detailed restriction

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* To whom correspondence should be addressed. Present address: Department of Genetics, Osaka University School of Medicine, Kita-ku, Osaka 530, Japan.

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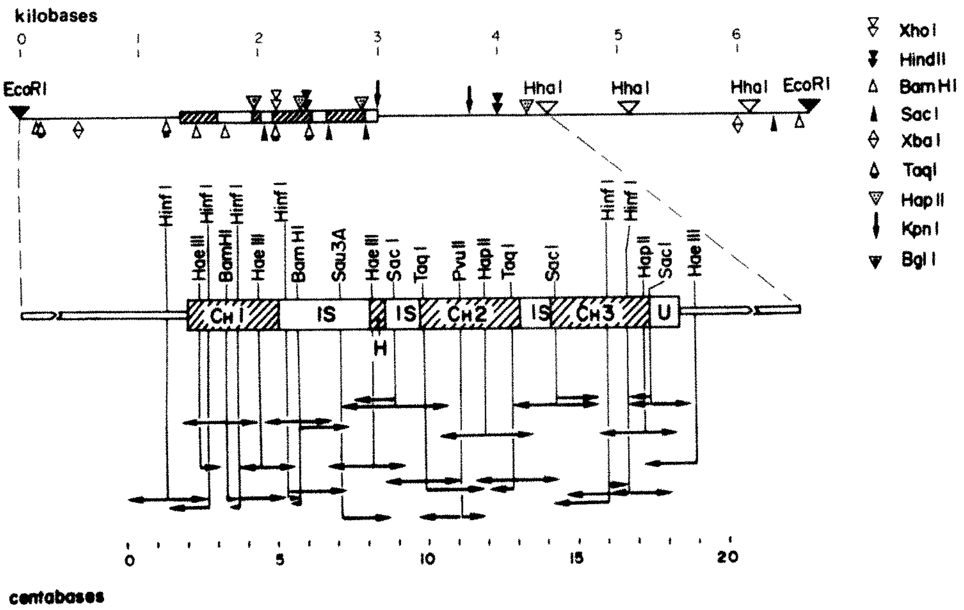

Fig. 1 Sequencing strategy of the mouse γ2b chain constant region gene. Plasmid DNA pIgH22 was purified according to the method of Clewell and Helinski[16]. Restriction cleavage sites were determined by combined cleavages of two or more enzymes. *Bam*HI, *Sac*I, *Xho*I and *Kpn*I sites were determined previously[3]. The 4.4-kilobase *Eco*Ri–*Hha*I fragment was isolated by 4% polyacrylamide gel electrophoresis after digestion of pIgH22 with *Eco*RI and *Hha*I, cleaved with other restriction enzymes and used as the substrates of phosphorylation. Alternatively, IgH22 was digested with either *Bam*HI or *Sac*I and resulting fragments were isolated. Each fragment was digested with the second restriction enzymes and used for phosphorylation. Sources of restriction enzymes were described previously[2]. The length of sequenced fragment is given in centabases in the 5′ to 3′ direction relative to the mRNA sequence. The horizontal arrows shown at the bottom indicate the direction and the extent of the sequence determined. The restriction map of the cloned 6.6-kilobase *Eco*RI fragment is presented on the top line. The restriction sites which were used for sequencing in the 4.4-kilobase *Eco*RI–*Hha*I fragment are shown on the rectangle below. The γ2b chain gene is represented by the wide rectangle. C_{H1}, C_{H2}, C_{H3}, domains of constant region; H, hinge region; IS, intervening sequence; U, untranslated sequence.

```
                         20                  40                  60                  80
0     GGAAATTTCTGACACTGCTTGCCTCAGATCAATTGTAGAAGACACGGTTCTAAGAGCAAAGCTAAGAACAGAATCTCTCCAAATATCCGAGGCCACTGAT

                                      A  K  T  T  P  P  S  V  Y  P  L  A  P  G  C  G  D  T  T  G  S  S  V
100   AAGAAAAAGCTCACACATTCTCCTCTCTTGCAGCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCTGGGTGTGGAGATACAACTGGTTCCTCCGT

        T  L  G  C  L  V  K  G  Y  F  P  E  S  V  T  V  T  W  N  S  G  S  L  S  S  S  V  H  T  F  P  A  L
200   GACTCTGGGATGCCTGGTCAAGGGCTACTTCCCTGAGTCAGTGACTGTGACTTGGAACTCTGGATCCCTGTCCAGCAGTGTGCACACCTTCCCAGCTCTC

        L  Q  S  G  L  Y  T  M  S  S  S  V  T  V  P  S  S  T  W  P  S  Q  T  V  T  C  S  V  A  H  P  A  S
300   CTGCAGTCTGGACTCTACACTATGAGCAGCTCAGTGACTGTCCCCTCCAGCACTTGGCCAAGTCAGACCGTCACCTGCAGCGTTGCTCACCCAGCCAGCA

       S  T  T  V  D  K  K  L
400   GCACCACGGTGGACAAAAAACTTGGTGAGAGGACATTCAGGGGAGGAGGGATTCACCAGAGTTGAGGCAAAGGTATTAGCCTATCTAAACCAGCCAGGCT
500   GGGATCCATCACCAAGGAGGTGACCTTAGCCCAGGGAAGAGGGAGATACTGTCTCTGCCTCCCTCCTGGGAACATCTAGCTATGACCACCTACACTCAAG
600   GACATGTTCCTCTGGGATAGGTGTGCTTGTCATTTCCAGGATCATCCTGGAACTAAGCCCATACCAGGACAAACTTTCCTCTCTCTGGTTTGGTGCTTCT
                                                                         I  N  P  C  P  P  C  K  E  C  H  K
                                                 E  P  S  G  P  I  S  T  I  N  P  C  P  P  C  K  E  C  H  K
700   CTCCTTCAAAAACCAGTAACATCCAGCCTTCTCTCTGCAGAGCCCAGCGGGCCCATTTCAACAATCAACCCCTGTCCTCCATGCAAGGAGTGTCACAAAT

       C  P
       C  P
800   GCCCAGGTAAGTCACTACCAGAGCTCCACTCCCAGGAGAATGGTAAGTGCTGTAAAAATCCCTGTAATGGAGGATAAGCCATGTACAAATCCATTTCCAT
                    A  P  N  L  E  G  G  P  L  V  F  I  F  P  P  N  I  K  D  V  L  M  I  S  L  T  P  K  V
                    A  P  N  L  E  G  G  P  S  V  F  I  F  P  P  N  I  K  D  V  L  M  I  S  L  T  P  K  V
900   CTCTCCTCATCAGCTCCTAACCTCGAGGGTGGACCATCCGTCTTCATCTTCCCTCCAAATATCAAGGATGTACTCATGATCTCCCTGACACCCAAGGTCA

      T  C  V  V  V  D  V  W  E  D  D  P  D  V  Q  I        F  V
      T  C  V  V  V  D  V  S  E  D  D  P  D  V  Q  I  S  W  F  V  N  N  V  E  V  H  T  A  Q  T  Q  T  H  R
1000  CGTGTGTGGTGGTGGATGTGAGCGAGGATGACCCAGACGTCCAGATCAGCTGGTTTGTGAACAACGTGGAAGTACACACAGCTCAGACACAAACCCATAG

                                                                         S  G  K  E  F  K  C  K  V  N  N  K
        E  D  Y  N  S  T  I  R  V  V  S  T  L  P  I  Q  H  Q  D  W  M  S  G  K  E  F  K  C  K  V  N  N  K
1100  AGAGGATTACAACAGTACTATCCGGGTGGTCAGCACCCTCCCCATCCAGCACCAGGACTGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAA

       D  L  P     P  I  E     T  I     K  I
       D  L  P  S  P  I  E  R  T  I  S  K  I  K
1200  GACCTCCCATCACCCATCGAGAGAACCATCTCAAAAATTAAAGGTGGGACCTGCAGGACAACTGCATGGGGGCTGGGATGGGCATAAGAATAAATGTCTA

                                                                 V
                                                           G  L  V  R  A  P  Q  V  Y  I  L  P  P  P  A
1300  TGTGGACAGCCTTCCACTTCAGCCATGACCTCTATGTATGTTTCTAACCCCACAGGGCTAGTCAGAGCTCCACAAGTATACATCTTGCCGCCACCAGCAG

      E  Q  L  S  R  K  D  V  S  L  T  C  L  V  V  G  F  N  P  G  D  I  S  V  E  W  T  S  N  G  H  T  E  E
1400  AGCAGTTGTCCAGGAAAGATGTCAGTCTCACTTGCCTGGTCGTGGGCTTCAACCCTGGAGACATCAGTGTGGAGTGGACCAGCAATGGGCATACAGAGGA

                                                                              K  T  S  K  W  E  K  T  D  S
        N  Y  K  D  T  A  P  V  L  D  S  D  G  S  Y  F  I  Y  S  K  L  N  M  K  T  S  K  W  E  K  T  D  S
1500  GAACTACAAGGACACCGCACCAGTCCTAGACTCTGACGGTTCTTACTTCATATATAGCAAGCTCAATATGAAAACAAGCAAGTGGGAGAAAACAGATTCC

        F  S  C  N  V  R  H  E  G  L  K  N  Y  Y  L  K  K  T  I  S  R  S  P  G
        F  S  C  N  V  R  H  E  G  L  K  N  Y  Y  L  K  K  T  I  S  R  S  P  G  K Term
1600  TTCTCATGCAACGTGAGACACGAGGGTCTGAAAAATTACTACCTGAAGAAGACCATCTCCCGGTCTCCGGGTAAATGAGCTCAGCACCCACAAAGCTCTC

                                                                            *poly(A)
1700  AGGTCCTAAGAGACACTGGCACCCATATCCATGCATCCCTTGTATAAATAAAGCATCCAGCAAAGCCTGGTACCATGTAAAACTGTCCTGGTTCTTTCCA

1800  AGGTATAGAGCATAGCTCACGGGCTGATGGGTCT
```

Fig. 2 Nucleotide sequence of mouse γ2b chain gene. The nucleotide sequence of the anti-coding strand is displayed 5′ to 3′. DNA sequencing was performed according to the method of Maxam and Gilbert[5]. DNA fragments to be sequenced were digested with the enzymes indicated in Fig. 1, treated with bacterial alkaline phosphatase (Boehringer) and phosphorylated using T4 polynucleotide kinase (New England Biolabs) and [γ-^{32}P]ATP (NEN). ^{32}P-DNA fragments were isolated by polyacrylamide gel electrophoresis and cleaved with second restriction enzymes. Fragments labelled only at one end were isolated by polyacrylamide gel electrophoresis and purified as described previously[2]. For strand separation the labelled DNA samples were dissolved in 100 μl of 0.2 M KOH, 10% (w/v) glycerol, 0.01% (w/v) xylene cyanol and 0.01% (w/v) bromphenol blue, heated at 37 °C for 1 min and separated with 15% or 20% polyacrylamide gel. Four base-specific reactions were utilised (G, A>C, C+T, C). Samples were loaded in 90% formamide and separated with thin gels (0.5 × 300 × 400 mm, 8 and 20% polyacrylamide gel). The amino acids predicted by the nucleotide sequence are shown above the coding sequences in italics. The partial amino acid sequences of MOPC 141[6] and MPC 11[7] are presented on the top line. Term, termination codon; poly (A), putative poly (A) addition site assigned by comparison with the mouse γ1 chain gene sequence. Amino acids are expressed by one letter code of A(Ala), C(Cys), D(Asp), E(Glu), F(Phe), G(Gly), H(His), I(Ile), K(Lys), L(Leu), M(Met), N(Asn), P(Pro), Q(Gln), R(Arg), S(Ser), T(Thr), V(Val), W(Trp) and Y(Tyr).

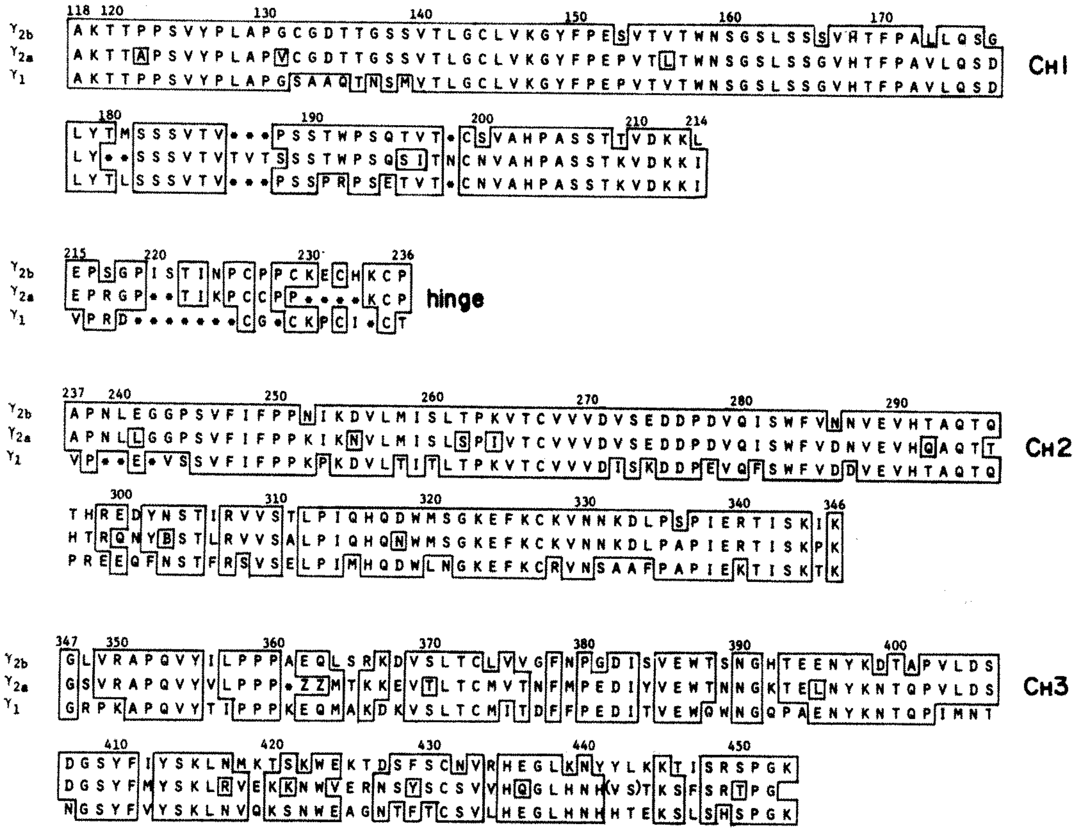

Fig. 3 Comparison of the amino acid sequences of γ1, γ2a and γ2b chains. The amino acid sequences of the γ1 and γ2b chains were predicted by the nucleotide sequences of the respective genes cloned from newborn mouse DNA[2]. The amino acid sequence of the γ2a chain was taken from the data of MOPC 173[7,8]. The residue was numbered with the first residue of C_{H1} domain as 118. Homologous positions are boxed. The one letter code used is as described in Fig. 2.

enzyme cleavage map of the *Eco*RI–*Hha*I fragment was constructed and the nucleotide sequence was determined by the chemical modification method of Maxam and Gilbert[5]. The restriction enzyme cleavage sites used for sequencing and the direction and range of sequencing are shown in Fig. 1.

The complete nucleotide sequence of the γ2b chain C region gene is shown in Fig. 2. The total number of the nucleotides determined is 1,834, which include the coding sequence, the IS, the 3′ untranslated sequence and the 5′ and 3′ flanking sequences.

The nucleotide sequence predicts the complete amino acid sequence of the C region of the γ2b chain (336 residues) which consists of the C_{H1} (residue 118–214), C_{H2} (residue 237–346) and C_{H3} domains (residue 347–453) and the hinge region (residue 215–236), respectively. The predicted sequence was compared with the partial amino acid sequences so far published, including 18 residues in the hinge region and the C_{H2} domain[6], 66 residues in the C_{H2} domain and 35 residues in the C_{H3} domain[7] (Fig. 2). The amino acid sequences derived from the nucleotide sequences matched the known amino acid

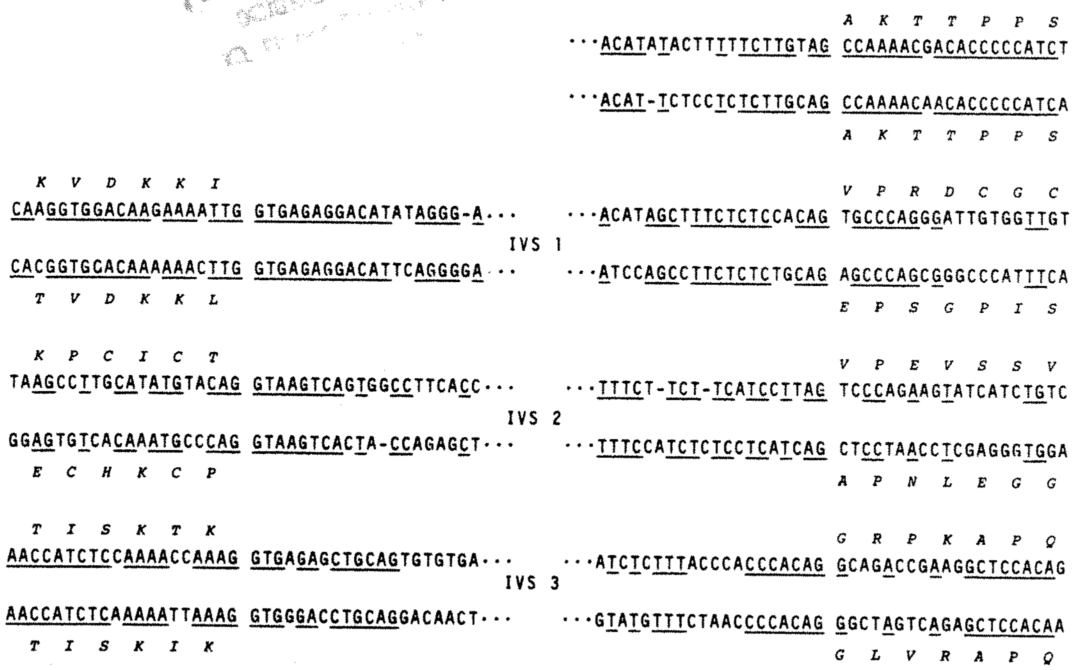

Fig. 4 Comparison of nucleotide sequences of the γ1 and γ2b chain genes around the junction of IS and coding sequences. Twenty nucleotides each of the homologous IS and coding sequences of the γ1 (upper sequences) and γ2b (lower sequences) chain genes are aligned to maximise homology. The top row is the 5′ end of the C_{H1} domain. The second, third and fourth rows are the 5′ and 3′ ends of the first, second and third IS, respectively. The nucleotide sequence of the strand corresponding to the mRNA is displayed 5′ to 3′ direction. The homologous nucleotides are underlined. Amino acids predicted from the nucleotide sequences are shown in italic letter by one letter code as described in Fig. 2.

sequences except two residues, Ser in place of Leu at residue 240 and Ser in place of Tyr at residue 268. To determine the junctions of the IS and coding sequences (see below) the nucleotide sequences were also correlated with the amino acid sequences of the γ2a chain[7,8] which share extensive homology with the known amino acid sequences of the γ2b chain[7].

The complete amino acid sequence of the C region of the γ2b chain is compared with the amino acid sequences of the γ1[2] and γ2a[7,8] chains as shown in Fig. 3. It is evident that the γ2b and γ2a chains show more homology to each other than to the γ1 chain. The results agree with our previous conclusion from cDNA–mRNA hybridisation studies[1]. The homology between the three γ chain subclasses (γ1, γ2a and γ2b) for each domain is calculated. The C_{H1} domains are most conserved (79–82%) throughout the three subclasses while the C_{H3} domains are least conserved (53–59%). The C_{H2} domains are highly conserved between the γ2a and γ2b chains whereas they are moderately conserved (63–68%) between the γ1 and γ2b (or γ2a) chains. The G + C content of the coding sequences is 52.3%, exceeding the value (41.8%) of the total mouse DNA[9].

Note that the nucleotide sequences predict an unexpected Lys residue at the carboxy terminus of the γ2b chain. Although most of the γ chains so far sequenced end with the Pro-Glys residues[10], the nucleotide sequences of the cDNA as well as chromosomal gene of the γ1 chain also predict an additional Lys residue at the end of the heavy chain[2,11]. These results suggest that the newly-synthesised γ2b chain proteins may have a Lys residue at the carboxy terminus, which will be removed eventually either at secretion or during circulation in the blood. It would be interesting to see whether the γ1 and γ2b chain proteins bound to the cellular surface have the Lys residue at their carboxy terminus since the amino acid sequencing was done using secreted immunoglobulins. The membrane-bound and secreted μ chains are reported to be different in the primary structures of their carboxy termini[12,13].

The comparison of the nucleotide sequences of the γ2b chain gene with the amino acid sequences of the γ2b and γ2a chains indicates that the domains and the hinge regions of the γ2b chain structural sequence are separated by three ISs as shown in Figs 1 and 2. The ISs split the γ2b chain gene at positions homologous to those in the γ1 chain gene[2,14]. The first, second and third ISs of the γ1 and γ2b chain genes as a pair are 356/316, 98/107, 120/112 base pairs long, respectively. The evolutionary significance of the ISs in immunoglobulin genes was discussed more extensively in previous reports[2,3].

The boundary sequences are compared between the γ1 and γ2b chain genes (Fig. 4). The first 10 nucleotides at each end of the ISs are highly conserved, although not identical, between the two genes whereas there is considerable divergence of the overall nucleotide sequences of the homologous IS. The possibility is discussed that not only nucleotide sequences at the joints of the IS and structural sequences but also other signals such as secondary and tertiary structures of mRNA precursors may be important for the recognition of splicing[15].

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A novel fragment of the corticotropin-β-lipotropin precursor

Rolf Håkanson*, Rolf Ekman†, Frank Sundler‡ & Roland Nilsson†

Departments of Pharmacology* and Histology‡, University of Lund, Lund, Sweden, and Department of Clinical Chemistry†, University of Lund at Malmö General Hospital, Malmö, Sweden

The pituitary ACTH/MSH cells are virtual storehouses for biologically important peptides. In addition to corticotropins, melanotropins, opioid peptides and lipolytic peptides these cells contain large amounts of at least three different polypeptides (or small proteins) having tryptophan in the amino-terminal position (Trp-peptides)[1–3]; their approximate molecular weight (MW) has been given as 14,000 (ref. 4). Nothing is known of their chemical properties and physiological significance. Experimental data indicate that the Trp-peptides in the ACTH cells are stored in the secretory granules[3] to be released jointly with ACTH and other secretory products, such as β-endorphin and/or β-lipotropin (β-LPH)[6]. ACTH and β-LPH are formed from a large common precursor protein where they constitute the carboxy-terminal portion[7–14]. Together the two peptides account for one-third to one-half of the molecular weight of the precursor and there has been considerable interest in the primary structure and possible physiological significance of the remaining amino-terminal portion[15]. The fact that the Trp-peptides occur in conspicuously high concentrations in the secretory granules of the ACTH/MSH cells prompted an investigation into their relation to the ACTH-β-LPH precursor protein. We have now isolated a glycopeptide with an apparent MW of ~11,000 residing in the secretory granules of the ACTH/MSH cells from pig pituitary. The amino acid sequence of its amino-terminal part was found to be identical with the amino-terminal end of the bovine corticotropin-β-LPH precursor protein[16]. We propose that the isolated new fragment is identical with the amino-terminal portion of the precursor that remains after ACTH and β-lipotropin have been split off.

Acetone powder was prepared from fresh frozen pig pituitaries and extracted with hot saline. The *p*H of the extract was adjusted to 3.9, the resulting precipitate was spun down and the supernatant was dialysed and freeze-dried (Table 1). The freeze-dried material was chromatographed on a DEAE-Sephadex column. The Trp-peptides could be separated into several peaks, the three major ones were labelled I, II and III (Fig. 1*a*). Each of the three Trp-peptides were further purified by gel chromatography (Fig. 1*b*). As a result, all of them appeared highly purified but only peptide I satisfied the criteria for complete homogeneity as judged by SDS-gel electrophoresis (Fig. 1*c*) and amino-terminal analysis. Tryptophan was identified as the amino-terminal amino acid (see below).

An antiserum was raised against peptide I. In a radioimmunoassay system this antiserum cross-reacted weakly with β-LPH and not at all with ACTH, α-MSH, β-MSH, β-endorphin or $\beta\text{-LPH}_{39-45}$. The antiserum was used to localise peptide I in the pituitary by immunohistochemistry. The results confirmed our previous fluorescence histochemical finding[3] that the Trp-peptide resides in the secretory granules of the ACTH/MSH cells (Fig. 2).

Peptide I was not recognised by antisera either to ACTH_{1-24} (ACTH radioimmunoassay kit, Amersham Radiochemical Centre) or to α-MSH (ACTH_{1-13}) which suggests that neither ACTH nor α-MSH is contained within its structure.

The amino acid sequence of the 1–35 portion of the amino-terminal end of peptide I was: Trp-Cys-Leu-Asp-Ser-Ser-Gln-Cys-Gln-Asp-Leu-Ser-Thr-Glu-(?)-Asn-Leu-Leu-Ala-Cys-Ile-Arg-Ala-Cys-(?)-Pro-Asp-Leu-Ser-Ala-Glu-Thr-Pro-Val-Phe. Comparison of the identified amino acid sequence with that of previously identified peptides and proteins showed it to correspond almost exactly to that recently predicted by Nakanishi *et al.*[16] for the amino acid sequence −105 to −71 of the ACTH-β-LPH precursor (Fig. 3). The prediction was based on nucleotide sequence analysis of cloned cDNA encoded by mRNA from bovine pituitary intermediate lobe. Our results differed from the prediction in position −102 where we found Asp instead of Glu, and in position −94 where we found Ser instead of Thr. These differences may be ascribed to the fact that the predicted amino acid sequence was based on a bovine nucleotide sequence whereas we have used porcine material. Since immunochemical analysis indicated that neither ACTH nor α-MSH are contained within the structure of peptide I and since the prediction has readily hydrolysable Lys-Arg in position −2 and −1 connecting with ACTH (ref. 17), it appears likely that the carboxy terminus corresponds to position −3 in the predicted ACTH-β-LPH precursor (Fig. 3). The amino acid composition of peptide I is given in Table 2. The presence of galactosamine and glucosamine residues suggests that peptide I is glycosylated. This finding is consistent with the observation that the ACTH-β-LPH precursor is a glycoprotein[18].

The remarkable similarities in amino acid sequence between the amino-terminal portion of peptide I and a part of the predicted ACTH-β-LPH precursor make it highly probable that peptide I is a fragment of the ACTH-β-LPH precursor. In addition, we propose that the isolated Trp-peptide exists as such in the secretory granules of the ACTH/MSH cells. It seems unlikely that the peptide is generated by cleavage at the Trp position during the process of purification, because specific fluorescence histochemical analysis has revealed the presence within these cells of extremely large amounts of polypeptides (or small proteins) having tryptophan at the amino terminus[1–3]. Thus, we believe that peptide I is a naturally occurring fragment of the ACTH-β-LPH precursor. The carboxy terminus of peptide I probably corresponds to position −3 in the ACTH-β-LPH precursor. If so, peptide I is the amino-terminal portion

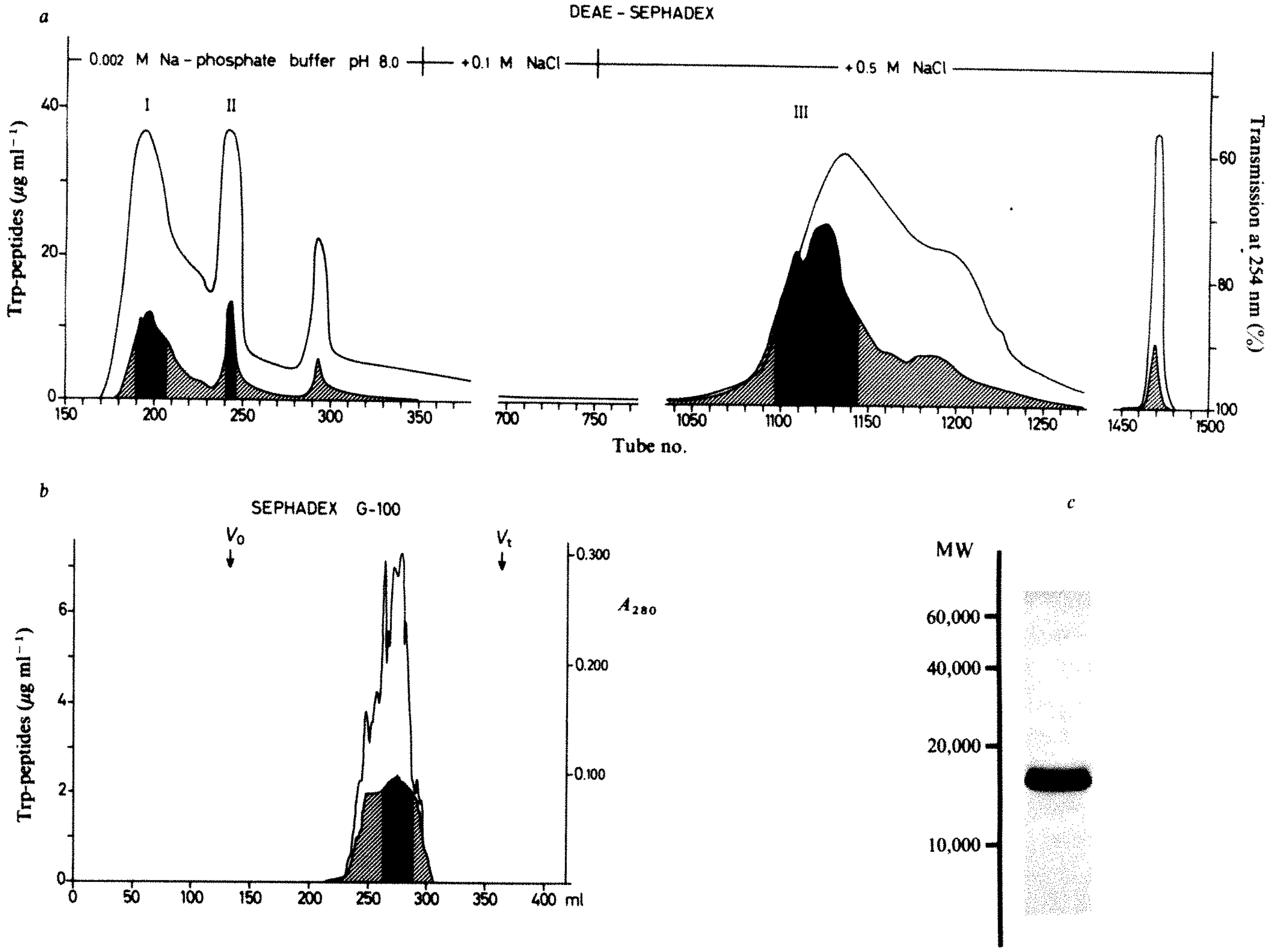

Fig. 1 *a*, Chromatography on a DEAE-Sephadex A-25 column (gel bed height 160 cm, diameter 5 cm) equilibrated with 0.002 M Na-phosphate buffer, *p*H 8.0. Freeze-dried extract of pig pituitaries (see Table 1) was dissolved in the buffer and applied to the column (3 g in 30 ml). The column was developed with the dilute phosphate buffer. Fractions of about 4 ml were collected. After the appearance of non-adsorbed proteins the elution buffer was enriched first with 0.1 M NaCl and then with 0.5 M NaCl. The stippled area shows the elution pattern of the Trp-peptides, measured fluorometrically[24]. The material collected for further purification is indicated in black. Peaks I, II and III were dialysed against redistilled water for at least 48 h and freeze-dried. The lyophilised material in peak I weighed 135 mg and was purified 5.3-fold compared with the acetone powder in Table 1. It was taken up in 0.1 M formic acid to a concentration of 50 mg ml^{-1} and applied to a Sephadex G-100 superfine column (gel bed height 90 cm, diameter 2.5 cm) equilibrated with 0.1 M formic acid, which was also used for elution (*b*). Fractions of 4 ml were collected and analysed for Trp-peptides. V_0 is the void volume defined by the use of blue dextran. V_t is the total mobile phase defined by the use of ^3H_2O. The weight of the purified material after dialysis and freeze-drying was 40 mg (Trp-peptide I). It was purified 6.2-fold. *c*, SDS-polyacrylamide (12.5%) gel electrophoresis of the isolated Trp-peptide (peptide I) was performed in a discontinuous system according to the method of King and Laemmli[30]. Samples were pretreated with 0.1 M 2-mercaptoethanol and heated at 90 °C in the presence of 1% SDS for 3 min. Separations were carried out in a Pharmacia apparatus GF-4 and proteins and peptides were stained with Coumassie brilliant blue R-250. The approximate molecular weight scale was deduced from the positions of nine standard proteins. The molecular weight of the isolated peptide appears to be over 15,000. This assessment, however, does not take into consideration the possibility that the peptide is rich in basic amino acids and/or glycosylated which may result in overestimation of the molecular weight.

Table 1 Recovery and yield of Trp-peptides in the initial purification steps

Preparation	Weight (g)	Protein (g)	Trp-peptides (mg)*	Calculated yield of Trp-peptides (%)	Relative purification† (-fold)
Acetone powder	225	140	150	100	1
Saline extraction (95–100 °C)	—	16	60	40	3.5
Precipitation at *p*H 3.9	13	10	50	33	4.7

One kg frozen pig pituitaries was homogenised in 10 l of cold acetone and left overnight. The resulting precipitate was washed with 40 l acetone and with 20 l acetone-diethyl ether (1 : 1) and finally with 20 l diethyl ether. The dried acetone powder was extracted with 4 l hot saline (95–100 °C) for 10 min. After cooling to 4 °C and centrifugation at low speed the *p*H of the supernatant was adjusted to 3.9 by the dropwise addition of 1 M HCl. After boiling for 3 min, the resulting precipitate was spun down and discarded, and the supernatant was dialysed against several changes of re-distilled water (at least 48 h) and freeze-dried. The concentration of Trp-peptides was determined fluorometrically with tryptophyl-alanine as standard[24]. Values are means of four separate runs. Protein was measured according to the method of Lowry *et al.*[25].

* Peptide weight expressed in terms of weight-equivalents of tryptophyl-alanine.

† Purification calculated from the ratio of μg Trp-peptides over mg protein.

that remains after β-LPH, ACTH, and the connecting Lys-Arg pair have been split off[17].

The prediction of the amino acid sequence of the ACTH-β-LPH precursor does not identify the amino terminus with certainty. Met in position −131 was considered a likely location for the translational initiation site[16]. We propose that the amino terminus of the precursor, following removal of the signal peptide, is Trp in position −105 (Fig. 3). It is probable that the amino acid sequence from −131 to −106 constitutes the signal peptide which is included in the primary translational product but lost upon packaging in the secretory granules. Hence, the granule-stored amino-terminal fragment of the porcine ACTH-β-LPH precursor consists of 103 amino acid residues and has a molecular weight of approximately 11,000 (Table 2).

The ACTH-β-LPH precursor (239 amino acid residues, MW ~26,000) generates β-LPH, ACTH and peptide I through enzymatic hydrolysis of consecutive basic residues[17]. Based on the occurrence of such consecutive basic residues within the precursor molecule other points of cleavage can be postulated.

Table 2 Amino acid composition of peptide 1

Residue	Residues determined*	Residues calculated to the nearest integer	Residues expected from predicted amino acid sequence −105 to −3
Lys	3.5	4	3
His	0.9	1	1
Arg	7.2	7	9
Asp	9.4	9	11
Thr	3.5	4	5
Ser	8.4	8	8
Glu	12.7	13	15
Pro	8.1	8	8
Gly	15.9	16	13
Ala	8.1	8	7
Half-cystine	6.6	7	4
Val	3.0	3	5
Met	1.1	1	1
Ile	1.4	1	1
Leu	7.1	7	6
Tyr	1.3	1	1
Phe	3.2	3	3
Trp†	1.5	2	2
Glucosamine	2.5	++	0
Galactosamine	0.6	+	0

Amino acid analyses were performed by hydrolysing samples of peptide I (100–200 μg) in 0.5 ml of boiling 5.7 M HCl at 110 °C for 24 and 72 h in sealed evacuated tubes. The hydrolysates were analysed according to the two column method of Spackman *et al.*[26] in a Durrum D-500 amino acid analyser. Carbohydrates were estimated on the hydrolysed samples. Half-cystine was determined as cysteic acid and methionine as methionine sulphone after performic acid oxidation[27]. Tryptophan was determined after hydrolysis in methanesulphonic acid[28] and before hydrolysis by a spectrophotometric method[29].

* All values are given in residues per 103 residues total amino acids (values for amino sugars and ammonia not included in summation). 103 is the number of residues expected from the predicted amino acid sequence −105 to −3. The calculated molecular weight of the peptide sequence is approximately 11,000.

† With both methods of determination the number of tryptophan residues was between 1 and 2.

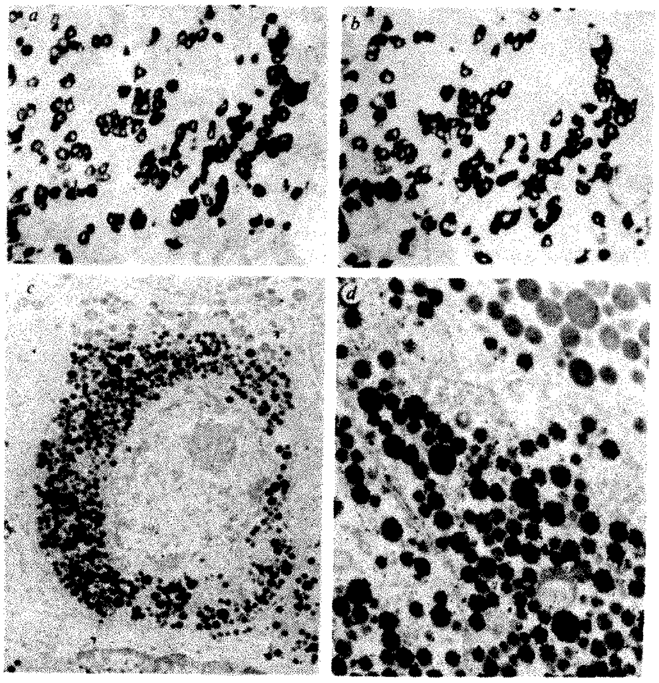

Fig. 2 Immunostaining of cells in the pig pituitary (anterior lobe) using antiserum to Trp-peptide I (*a*) and ACTH (ref. 3) (*b*). Three rabbits were immunised with peptide I by subcutaneous injection (multiple site) of the antigen (200 μg) emulsified with 1 ml of Freund's adjuvant. Booster injections were given 4 weeks later. Satisfactory antibody titre for radioimmunoassay (1 : 1,000) was obtained in one of the rabbits 2 weeks after the second injection. The cells displaying peptide I immunoreactivity were identical with the ACTH cells as shown by immunostaining consecutive plastic sections. Formaldehyde-fixed tissue was embedded in Epon. Sections, 1-μm thick, were exposed to either of the two antisera (each in dilution 1 : 320) for 24 h at 4 °C. The site of antigen-antibody reaction was identified by the peroxidase-antiperoxidase method of Sternberger[31]. The intracellular storage site of peptide I was established by electron immunocytochemistry (*c*, *d*). Note intense immunostaining over all secretory granules of the ACTH cell. Non-reacting cell (probably GH cell) in upper right corner of *d*. (*a* and *b* ×220; *c* ×3,100; *d* ×9,900.)

ACTH may yield α-MSH (ACTH_{1-13}) and CLIP (ACTH_{18-39}). β-LPH yields γ-LPH ($\beta\text{-LPH}_{1-58}$) and β-endorphin ($\beta\text{-LPH}_{61-91}$). The amino-terminal part of the precursor (peptide I) may yield a smaller Trp-peptide (residues −103 to −58), γ-MSH (residues −56 to −43) and a carboxy-terminal fragment (residues −41 to −1) (Fig. 4).

The fact that the isolated amino-terminal part of the ACTH-β-LPH precursor exists as such in the secretory granules of the ACTH/MSH cells and that it is released together with other secretory products upon stimulation[6] makes it tempting to speculate that it exerts a physiological function in the circulation. Some preliminary tests of bioactivity have been performed. The melanotropic activity, tested in a bioassay system[19], was such that 1 μg of the Trp-peptide gave the same response as 3.4×10^{-3} μg α-MSH (Sigma). This probably reflects the His-Phe-Arg-Trp sequence (−51 to −48) which is known to carry melanotropic activity[20]. The peptide had weak lipolytic activity on isolated rabbit adipocytes[21]; 1 μg of the peptide gave about the same response as 10^{-2} μg β-LPH. The peptide had no

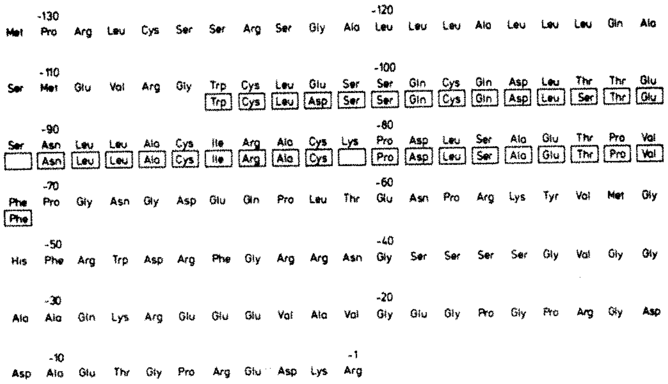
-130
-120
Met Pro Arg Leu Cys Ser Ser Arg Ser Gly Ala Leu Leu Leu Ala Leu Leu Leu Gln Ala
-110
-100
Ser Met Glu Val Arg Gly Trp Cys Leu Glu Ser Ser Gln Cys Gln Asp Leu Thr Thr Glu
Trp Cys Leu Asp Ser Ser Gln Cys Gln Asp Leu Ser Thr Glu
-90
-80
Ser Asn Leu Leu Ala Cys Ile Arg Ala Cys Lys Pro Asp Leu Ser Ala Glu Thr Pro Val
Asn Leu Leu Ala Cys Ile Arg Ala Cys Pro Asp Leu Ser Ala Glu Thr Pro Val
-70
-60
Phe Pro Gly Asn Gly Asp Glu Gln Pro Leu Thr Glu Asn Pro Arg Lys Tyr Val Met Gly
Phe
-50
-40
His Phe Arg Trp Asp Arg Phe Gly Arg Arg Asn Gly Ser Ser Ser Ser Gly Val Gly Gly
-30
-20
Ala Ala Gln Lys Arg Glu Glu Glu Val Ala Val Gly Glu Gly Pro Gly Pro Arg Gly Asp
-10
-1
Asp Ala Glu Thr Gly Pro Arg Glu Asp Lys Arg

Fig. 3 The predicted amino acid sequence of the amino-terminal portion of the bovine ACTH-β-LPH percursor after ACTH and β-LPH have been split off (according to Nakanishi *et al.*[16]). The amino acid residues are numbered in the direction from amino terminus to carboxyl terminus, with the first residue proximal to ACTH given as no. −1; being on the NH_2-terminal side of ACTH all the residues are indicated by negative numbers (see ref. 16). Amino acid residues in boxes represent the identified primary structure of the Trp-peptide isolated from pig pituitary. Amino acid sequencing[32] was made with a Beckman sequencer (model 890 C) using the standard Beckman 1 M Quadrol program (Beckman 122974) with polybrene to retain the peptide[33,34]. Phenylthiohydantoin (PTH) derivates were identified by high-pressure liquid chromatography on a 30-cm Waters μ-Bondapak C18 column by using stepwise elution with a system with methanol-containing buffers (Waters Associates) for the ethylacetate phases and a 60/40 mixture of methanol and 0.01 M sodium acetate for the aqueous phases. TLC on fluorescent silica gel plates was used as an additional method of identification[35]. Moreover, PTH-carboxy-methylcysteine residues were identified by radioactive labelling.

adrenocorticotropic activity when tested *in vivo*[22], and it did not cause displacement of naloxone in an opiate receptor-ligand assay[23]. The functional significance of peptide I and of the other pituitary Trp-peptides (which may well be fragments of peptide I) remains to be established.

After submission of this paper, Keutmann *et al.*[36] described the partial purification from mouse pituitary tumor cells of a glycoprotein accounting for most of the non-ACTH, non-β-LPH region of the ACTH-β-LPH precursor. The amino terminus was found to be tryptophan.

The sequencing data are available from the authors on request. We thank Dr David Eaker for determining the amino acid composition, Dr Leonard Larsson for performing the assay of melanotropic activity, Dr Per Melin (Ferring AB) for performing the assay of adrenocorticotropic activity and Professor P. Schwandt for assaying the lipolytic acitivity. Antiserum against α-MSH was a gift from Dr D. Swaab. The investigation was supported by grants from the Swedish Medical Research Council (04X-1007) and from the Medical Faculty of Lund, Lund, Sweden.

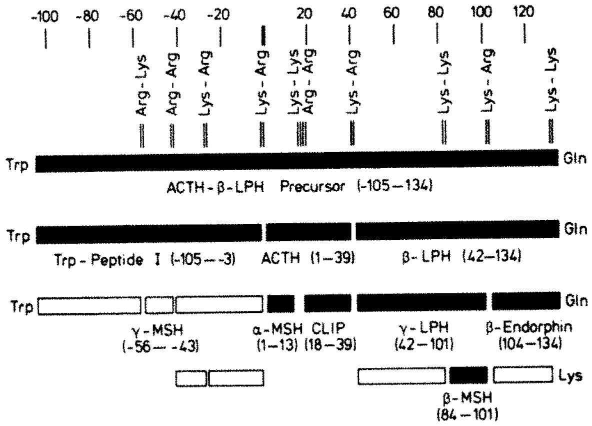

Fig. 4 The proposed ACTH-β-LPH precursor protein (putative signal peptide not included) and the various fragments that may be generated from the precursor by proteolytic processing, assuming a protease specific for consecutive basic residues[17] acting on the many pairs of Arg or Lys (indicated in the figure) connecting the component peptides in the precursor molecule. Identified or postulated fragments are shown in black in contrast to purely hypothetical fragments.

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Recovery frequency of phages λ and M13 from human and animal faeces

Susan A. Schluederberg, Bonnie Marshall, Chikanori Tachibana & Stuart B. Levy

Tufts University School of Medicine, Boston, Massachusetts 02111

Derivatives of bacteriophages λ and M13 are in common use as vectors in recombinant DNA research[1–5]. These laboratory-derived phages have been designed to allow cloning of DNA fragments, but to be unable to survive outside a defined laboratory and/or host-cell environment. To assess the availability of wild-type λ or M13 phages in the environment which might potentially rescue debilitated derivative phages, we have now examined the frequency of these and other bacteriophages in human and animal faeces. We detected coliphage in over two-thirds of the faecal samples. Of these, 1.2% of the samples contained λ-like phage and 3.5% had phage indistinguishable from M13.

Bacteriophage λ was originally discovered as a prophage in *Escherichia coli* K12 by Lederberg[6]. It has been the subject of extensive genetic and molecular studies. The Charon phages 3A, 4A and 16A are derivatives of λ which have been certified for recombinant DNA studies in an EK2 system[7] when they are grown in debilitated *E. coli* K12 DP50 or DP50 *supF* (ref. 1).

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Another derivative of phage λ, λ·gt WES B (refs 2, 3), was produced by introducing amber mutations and other modifications into phage λ·gt·λC (ref. 4). It has also been certified as an EK2 vector when grown in *E. coli* K12 DP50 supF. M13 is a male-specific DNA phage originally isolated by Hofschneider[8] from a strain of *E. coli*. Derivatives of this phage have been produced for use as cloning vehicles[5].

Table 1 *E. coli* strains studied

Strain	Relevant characteristics	Source
D0-27	*E. coli* $C\lambda^s$, F^-	M. Malamy
D0-28	*E. coli* $C\lambda^s$, F^- nal^R	This laboratory*
D0-29	*E. coli* $C\lambda^R$ mal^- F^- nal^R	This laboratory†
B0-11	*E. coli* $C\lambda^R$ nal^R F^+	This laboratory
U-7	*E. coli* K12 HFr (λ^+)	M. Malamy

* Nalidixic acid resistant mutants of D0-27 were isolated without mutagenesis after heavy inoculate plating on MacConkey agar containing 30 $\mu g\ ml^{-1}$ nalidixic acid.

† A spontaneous, λ resistant mal^- derivative of D0-28. Resistance to λ was confirmed by inability of λ^{vir} to grow.

In our study, four populations of humans were sampled—hospital patients, personnel from clinical and research laboratories, first-year medical students living in the Boston area (urban), and people residing in Sherborn, Massachusetts (rural). Animals from a farm in Sherborn, 20 miles west of Boston, were also sampled. Faecal specimens were collected on sterile 'Culturettes' (Scientific Products) and streaked onto MacConkey plates. Phage assays were carried out as described in Table 2 using strains listed in Table 1. During the initial screening process, the presence of phage λ was detected by identifying those plaques on strain D0-28 (*E. coli* $C\lambda^s$, F^-) which did not appear on strain D0-29 (*E. coli* $C\lambda^r$, F^-). The presence of M13 was detected by its ability to form plaques on strain B0-11 (*E. coli* C, F^+), but not on D0-29.

Among the 303 samples tested in both studies, phage were detected at an average frequency of 69% (Table 2). The highest percentage of samples with phage was found in the urban and laboratory groups. This finding agrees with a recent report[9] that urban sewage yielded a higher concentration of phages than rural sewage. Phage isolates suspected of being λ were tested for DNA homology with known λ phage using DNA·DNA filter hybridisation[10] to ^{32}P-labelled λ55 (ref. 11). In addition, the plaque isolates were tested for their ability productively to infect λ-lysogenic *E. coli*. Homoimmune λ-like phages should produce no plaques on λ-lysogenic *E. coli* due to the immunity conferred on lysogenic cells by the λ repressor. By the above criteria, only 2 of the 162 samples (frequency 1.2%) contained bacteriophage which were indistinguishable from λ. They came from a rural housewife and one of the laboratory personnel.

The M13 isolates formed turbid plaques on the F^+ indicator strain (B0-11), but no plaques on the F^- strain (D0-28). To confirm that these isolates were M13, each was hybridised[10] to a ^{32}P-labelled RF M13. This probe was made by nick translation[12] of RF DNA. Additional confirmation that the plaques were M13 came from agarose gel electrophoresis of supernatants from phage-infected cells in which the DNA was easily detected by ethidium bromide staining of the gels (L. Laverne, J. Hines and D. Ray, personal communication). Each M13 isolate collected from a productive infection of B0-11 showed a mobility identical to native M13 (Fig. 1). Furthermore, suspected M13 phages were neutralised by antiserum directed against the M13 coat protein.

M13-like phages were recovered in 5 out of the 141 samples (3.5%) from humans. The highest frequency was found in hospital (9.1%) and rural (farm) (5.9%) samples. M13 was detected at the same frequency (5.9%) in samples from farm mammals, but was not recovered in chicken or fly samples from the same farm.

Our findings provide the first data regarding frequency of λ and M13 in man's immediate environment. As these phages naturally infect *E. coli*, their presence would be most evident in faecal samples. In this series of tests, both phages were easily found, but at relatively low frequencies. Our screening method identified wild-type phages with receptors (tail fibres) similar to λ or M13. However, it would not have detected M13-like and λ-like phages having regions of DNA homology and potential rescue ability, but different receptors. Thus heteroimmune lambdoid phages, such as 21, 82, 424 and 434 (ref. 13), would have been detected, but not ϕ80 (ref. 14) which has a different receptor.

The frequency of M13 has not previously been assayed. Dhillon *et al.*[9], assaying Hong Kong sewage, found 24 male-

Table 2 Frequency of phage isolation from faecal samples in different environments

Human samples	No. of samples tested for λ	% Samples with any phage	% Samples with λ-like phage	No. of samples tested for M13	% Samples with any phage	% Samples with M13
Hospital	50	44.0	0	33	60.6	9.1
Laboratory	39	84.6	2.6	48	75.0	0
Urban	48	89.6	0	43	72.1	2.3
Rural	25	68.0	4.0	17	41.2	5.9
Total	162	71.0	1.2	141	66.7	3.5
Non-human samples						
Flies	4	75.0	0	7	28.6	0
Chickens	4	75.0	0	5	60.0	0
Mammals*	9	100.0	0	17	64.7	5.9
Total	17	88.2	0	29	55.2	3.5

For assay of λ phage, faecal samples were plated onto MacConkey plates and incubated overnight at 37 °C. These samples were then replica-plated onto plates (Luria + 1 mM $MgSO_4$ + 0.2% maltose plates) containing newly inoculated lawns of logarithmically growing D0-28. As a positive control, a plate of *E. coli* U-7 (λ^+) was processed in the same manner as a test sample. After overnight incubation at 37 °C, the phages were collected by suspending the plate contents in 4 ml cold ϕ80 buffer. Cells were removed by pelleting in a clinical centrifuge. The resultant supernatants were diluted in ϕ80 buffer and assayed for phage using D0-28 and D0-29 indicator strains in soft agar overlays on nalidixic acid 30 $\mu g\ ml^{-1}$. For further testing, plaques were probed with microcapillary pipettes and resuspended in 0.5 ml ϕ80 buffer. For M13 phage assay, several loops of faecal bacteria from each sample MacConkey plate were used to inoculate 5-ml log phase cultures of B0-11 in TYE broth. This strain was used to enrich the male-specific phages. A culture of B0-11 inoculated with M13 was the positive control. Cultures were shaken overnight at 37 °C. Supernatants were obtained by sedimenting cells in a clinical centrifuge. Indicator cells for titration were grown to late logarithmic phase, sedimented and resuspended in an equal volume of 0.1 mM NaCl and shaken for 20 min. Cells were then pelleted and resuspended in 0.1 mM NaCl. Dilutions of supernatants in TE (10 mM Tris-HCl, pH 8.0 1 mM EDTA) buffer were plated with F^+ and F^- indicator cells in Trypticase medium soft agar overlays.

* Cattle, horses, cats, dogs, goats and pigs.

specific phages amongst the 77 phage isolates studied. Nine of the male-specific phages, 11.7% of the isolates, were considered to be single-stranded DNA phages by their 'turbid' plaque morphology and were inactivated by anti-AE_2 serum.

In a study of 500 strains of human *E. coli*, Jacob and Wollman[13] found only three isolates which were indistinguishable from λ by immunity, host range and antiserum neutralisation. Fourteen other temperate phages of unique immunity types shared some, but not all, characteristics with λ. Our findings appear similar to those of Jacob and Wollman, published in 1956.

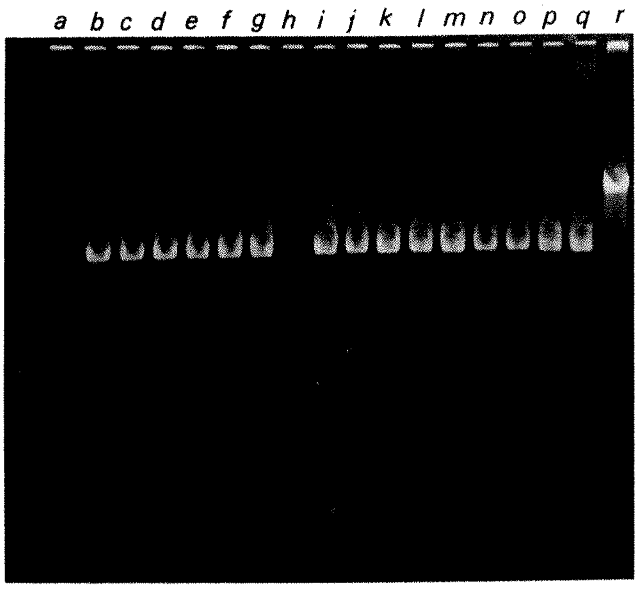

Fig. 1 Agarose gel (0.8%, 1.5 mm) electrophoresis in Tris-borate-EDTA buffer (*p*H 8.3) of supernatants from B0-11 cultures infected with plaque isolates. Aliquots of 30 μl of supernatants from phage-infected B0-11 cultures were added to 3 μl 2% SDS, 3 μl 0.25 M EDTA and 4 μl 0.1% BPB–50% glycerol and incubated at 65 °C for 10 min. Samples were then applied to the gel and analysed after electrophoresis for 3 h at 25 mA. Negative controls (*a*, *h*) were from two separate cultures of B0-11 cells alone. Positive controls (*f*, *l* and *q*) were native M13. Phage λ was applied to slot *r*. All other samples were phage isolates.

Our results can be used in assessing the potential for interaction of λ or M13 cloning vectors with wild-type phages in man's environment.

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Host range of *Agrobacterium tumefaciens* is determined by the Ti plasmid

M. F. Thomashow*, C. G. Panagopoulos†,
M. P. Gordon‡ & E. W. Nester*

* Department of Microbiology and Immunology, University of Washington, Seattle, Washington 98195
† Institut Phytopathologique Benaki, Kiphissia, Athens, Greece
‡ Department of Biochemistry, University of Washington, Seattle, Washington 98195

The plant pathogen *Agrobacterium tumefaciens* can induce crown gall tumours in a wide range of dicotyledonous plants. To be virulent an *Agrobacterium* strain must contain a tumour-inducing (Ti) plasmid[1,2]. Most Ti plasmids fall into one of two groups, based on whether they code for enzymes for octopine or nopaline utilisation[3,4]. Tumours induced by strains carrying octopine or nopaline type Tiplasmids generally synthesise octopine or nopaline respectively[3,4]. Normal plant tissues do not synthesise these unusual amino acid derivatives[5,6]. Chilton *et al.*[7] have analysed total DNA isolated from a cloned sterile tobacco crown gall callus and found sequences (T-DNA) complementary to ~5% of the Ti plasmid that are not found in normal tobacco callus. Thus, *Agrobacterium* can induce and maintain tumours by introducing genetic information stably into plant cells. It is evident that plasmid sequences have a central role in *Agrobacterium* virulence. Based on metabolic and physiological properties, most isolates of *A. tumefaciens* can be classified as either biotype 1 or biotype 2 organisms[8,9]. They have a broad host range, inducing tumours on most dicotyledonous plants. Recently, however, strains isolated in Greece and Russia from grapevine tumours have been shown to have a limited host range[10,11]. These organisms are also unique in that they do not fit into either of the previously described biotypes and have therefore been assigned to a new class, biotype 3 (refs 11, 12). We felt that these limited host range organisms could provide a system to study determinants of host range: if host range is determined solely by plasmid genes, the transfer of the Ti plasmid from a limited host range biotype 3 organism (assuming the biotype 3 organism had a Ti plasmid) to a biotype 1 organism that had been cured of its Ti plasmid, should yield a transformant of limited host range. The transformant should have a wide host range if this is a chromosomally borne trait. In this report we present data suggesting that the Ti plasmid has a major role in determining host range.

A. tumefaciens strains Ag57 and Ag63 isolated in Greece, and Ag158 and Ag162 isolated in Russia are biotype 3 organisms with a limited host range. Plasmids from these strains were isolated and subjected to electrophoresis through an agarose gel (Fig. 1). All of the isolates contained two large plasmids with molecular weights (MWs) of $\sim 140\times10^6$ and 130×10^6. Both Ag63 and Ag158 contained an additional plasmid with MWs of $\sim 65\times10^6$ and 72×10^6, respectively. Strain Ag162 contained two additional plasmids with MWs of about 55×10^6 and 28×10^6. Plasmids from the four strains were isolated and used to transform strain A136 as previously described[13]. This latter strain is a biotype 1 organism that has been cured of its virulence plasmid pTiC58 but retains a cryptic plasmid pATC58 (ref. 14, see Fig. 1). Transformants were selected on a minimal medium with octopine added (400 μg ml^{-1}) as the sole source of carbon and nitrogen. All four donor strains can catabolise octopine (M.F.T. and E.W.N., unpublished results; C.G.P., unpublished results) and we hoped that, as with biotype 1 organisms, this characteristic would be a

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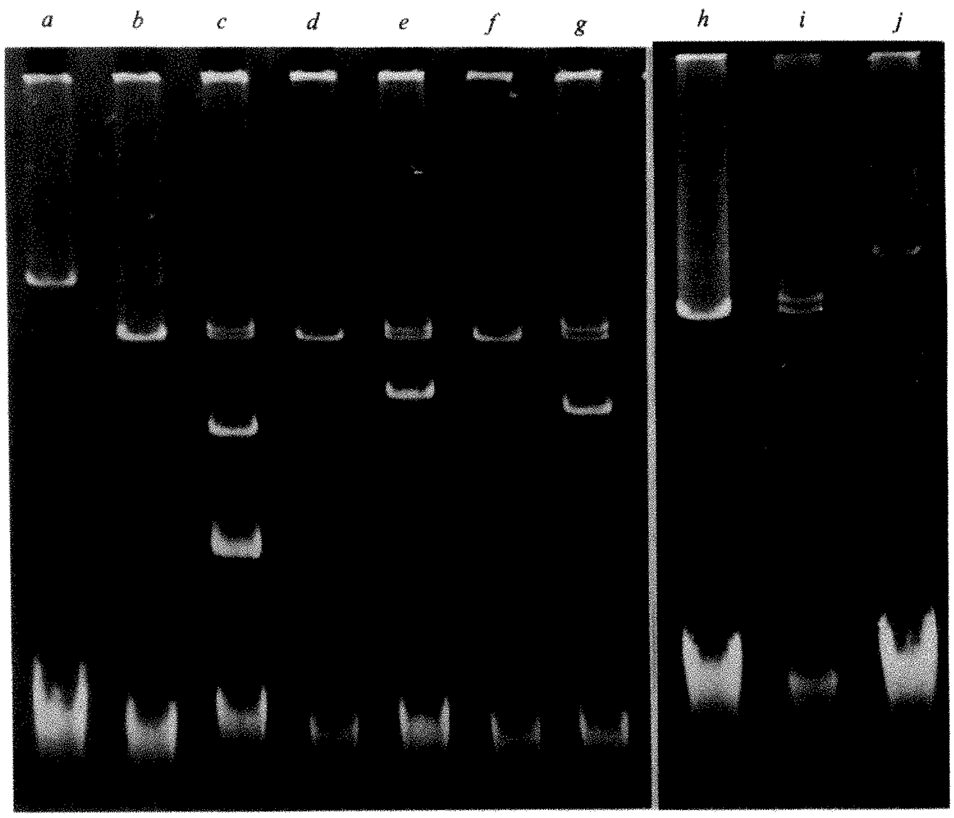

Fig. 1 Plasmids from *A. tumefaciens* strains with a limited host range and their respective transformants. Plasmid was isolated by a modified version of the Currier–Nester technique[24] as described by Casse *et al.*[14] Samples were subjected to electrophoresis through a 0.7% agarose gel as previously described[7] except that a vertical gel apparatus was used. Plasmid bands were visualised by staining with ethidium bromide (1 μg ml^{-1}) and illuminating with a Blak-Ray Transilluminator (UV Products). Samples are as follows: *a* and *j*, A136; *b*, Ag162Tr; *c*, Ag162; *d*, Ag158Tr; *e*, Ag158; *f*, Ag63Tr; *g*, Ag63; *h*, Ag57Tr; *i*, Ag57.

plasmid-borne trait. This proved to be true as octopine-utilising transformants were recovered. They were typical biotype 1 organisms in that they were 3-ketolactose positive and grew on the selective medium of Schroth *et al.*[15]. In each case the transformants (Tr) contained the 130 × 10^6 MW plasmid found in the parental strain (Fig. 1). When plasmids from the parents and transformants were digested with the restriction endonuclease *Sma*I and the products separated by electrophoresis on an agarose gel, restriction fragments of the plasmids from the transformants formed a subset of the restriction bands found in their respective parents (data not shown).

The *Bst*I restriction patterns of the plasmids from the transformants are presented in Fig. 2. The pattern of the pATC58 plasmid is not visible because its relative yield in the plasmid preparations is low (see Fig. 1). The patterns of the transformants are strikingly similar, suggesting that the plasmids are closely related. The banding patterns of the plasmids contained in Ag158Tr and Ag162Tr actually appear identical although their respective parents are obviously different (Fig. 1). Their pattern differs by only one band from that obtained with the plasmid from Ag63Tr. However, the restriction patterns of the plasmids in the transformants bear little resemblance to that of the pTi-B6-806 plasmid, the octopine Ti plasmid present in the biotype 1 strain A277. This is of interest because the restriction patterns of all octopine type Ti plasmids investigated to date have been virtually identical[16]. This is exemplified by the *Bst*I digestion patterns of two typical octopine plasmids, pTi-A6 and pTi-15955 (Fig. 2). These data suggest that octopine plasmids present in limited host range strains form a family of plasmids distinct from the wide host range octopine plasmids.

The host range of strains Ag57, Ag63, Ag158, Ag162 and their transformants is presented in Table 1. The virulence response of the transformant was virtually identical to the donor strain with all host plants tested. Significantly, when the donor strain gave an attenuated virulence response, as seen with Ag57 and Ag63 on sunflower and *Datura*, the transformants gave the same response. With tobacco, a slight difference in virulence between the limited host range donor strains and their transformants is suggested. Both Ag57 and Ag63 formed small,

Table 1 Virulence of *Agrobacterium* strains on various host plants

Bacterial strain*	Host plants						
	Grapevine *Vitis vinifera* c.v. Sultanina	Tomato, c.v. San Pietro	*Kalanchoë diagremontiana*	*Datura*	Sunflower	Tobacco (*Nicotiana tabacum* c.v. Turkish)	Tobacco (*N. glutinosa*)
Ag57	+	–	–	–/(+)	–/(+)	(+)	ND
Ag57Tr	+	–	–	–/(+)	–/(+)	–	ND
Ag63	+	–	–	–/(+)	–/(+)	(+)	ND
Ag63Tr	+	–	–	–/(+)	–/(+)	–†	ND
Ag158	+	–	ND	–	–	–	+
Ag158Tr	+	–	ND	–	ND	–	+
Ag162	+	–	ND	–	ND	–	+
Ag162Tr	+	–	ND	–	ND	–	+
A277	–	+	+	+	+	+	+
A136	–	–	–	–	–	–	–

The symbols used to describe the virulence response are; +, large active galls; (+), small galls that appear late compared with A277; –/(+), small galls that appear late and not at every inoculation site; –, no gall formation; ND, not determined.

* Strains Ag57, Ag63, Ag158, Ag162 and their transformants (Tr) are described in the text. The derivation of strains A136 and A277 have been described previously[2,16].

† This strain was avirulent on *Nicotiana tabacum* c.v. Turkish but gave very small, late appearing galls on *Nicotiana tabacum* c.v. xanthi.

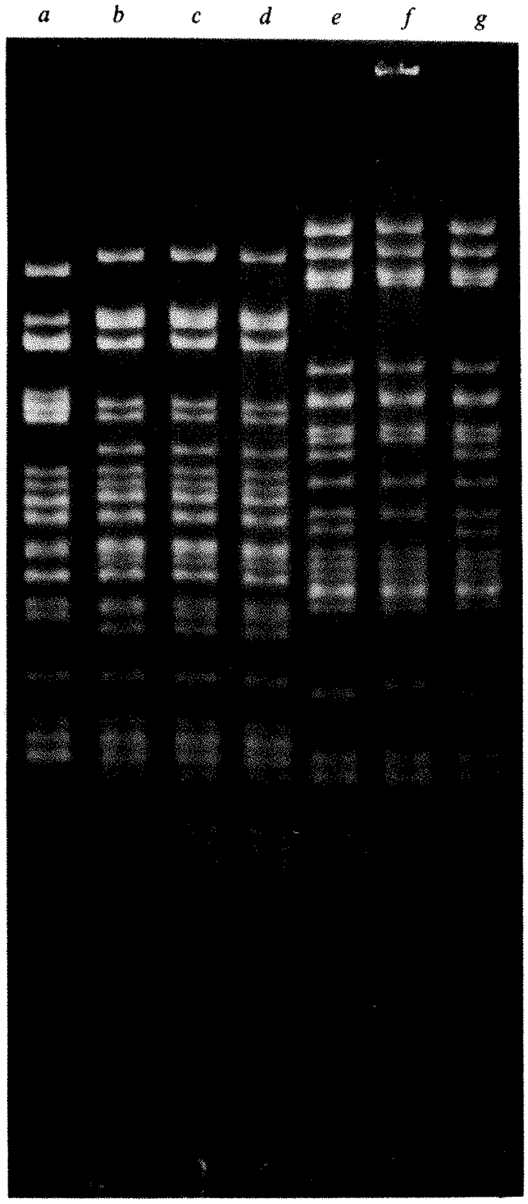

Fig. 2 *Bst*I cleavage patterns of isolated plasmids. Plasmid (0.5 μg) isolated by the Currier–Nester procedure[24] was digested with *Bst*I at 37 °C in a buffer consisting of 100 mM Tris-HCl (*p*H 7.6), 6 mm $MgCl_2$ and 100 mM NaCl. The products were separated by electrophoresis through a 0.7% agarose gel as previously described[7]. The restriction bands were visualised as in Fig. 1. Plasmid was isolated from the following strains: *a*, Ag57Tr; *b*, Ag63Tr; *c*, Ag158Tr; *d*, Ag162Tr; *e*, A277; *f*, A6; *g*, 15955.

slowly developing galls on *Nicotiana tabacum* c.v. Turkish, while the transformants formed no galls at all. Ag63Tr did form very small galls on *Nicotiana tabacum* c.v. xanthi. The data also show that although A277 has a wide host range, it is not virulent on grapevine. This inability to induce galls on grapevine must be a function of the Ti plasmid and not the chromosomal DNA as all the limited host range transformants formed large active galls on grapevine. It is clear from these data that the major determinant of host range for the strains tested is plasmid coded. The results obtained with tobacco suggest that chromosomal DNA, or possibly the other plasmids harboured by Ag57 and Ag63 might modulate host range to a limited extent.

How plasmid genes determine host range is not known. Apparently, effective binding of agrobacteria to plant cells is a prerequisite to tumour formation[17,18]. *A. tumefaciens* strains containing Ti plasmids bind more effectively to tobacco and carrot cells than do strains that do not harbour Ti plasmids[19,20]. These data suggest that it is the Ti plasmid that enables *Agrobacterium* to bind to plant cells. It is possible that the Ti plasmids from the limited and wide host range isolates allow *Agrobacterium* to bind to cells of different plant species. An analogous system for enteropathogenic *Escherichia coli* has been shown to exist. Plasmid sequences code for surface antigens that allow the bacteria to bind to animal cells; different antigens allow binding to cells from different animal species[21]. Without binding, *E. coli* strains that are able to produce enterotoxin are not pathogenic[22].

An additional ancillary conclusion that may be drawn from these data is that all octopine Ti plasmids do not form a homogeneous group as previously thought[16]. Current studies in our laboratories are aimed at determining the extent to which these and other limited host range octopine Ti plasmids differ from their wide host range counterparts.

Since submission of this letter we have transferred a wide host range Ti plasmid into strain Ag63. The transconjugant assumes the wide host range of the donor strain (V. Knauf and A. Montoya, unpublished observations). Our data on the importance of the plasmid in determining host range agree with the conclusions of Loper and Kado[23].

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Erratum

In the News Feature 'Recombinant DNA: have recent experiments assessed all the risks?', *Nature* **282,** 773, 1979, column 3 lines 5–7 on page 773 should read ';intact recombinant λ or plasmid DNAs with single polyoma inserts gave variously 0, 6 and 9% tumorigenicity'. (Not 0.6 and 9%.)

BOOK REVIEWS

Other men's minds

Kevin Connolly

The Making of Mind: A Personal Account of Soviet Psychology. By A.R. Luria. Edited by Michael Cole and Sheila Cole. Pp.234. (Harvard University Press: Cambridge, Massachusetts, and London, 1979.) $15; £9.

"PEOPLE come and go, but the creative sources of great historial events and the important ideas and deeds remain." This sentiment, expressed in the penultimate sentence of Alexander Romanovich Luria's short autobiography gives the key to the book. It is one of that small genre of intellectual autobiographies; it is fascinating because of the man and his work, and important because it spans almost the whole of Soviet psychology as it emerged from the deep and frowsty conservatism of tsarist Russia after the October revolution.

Luria was born in Kazan in 1902, into the Russian intelligentsia class. Arguably he was the best known Russian psychologist of his day, a prolific writer and an inveterate traveller. The cataclysmic changes of 1917 had a profound effect upon him: "The revolution freed us, especially the younger generation, to discuss new ideas, new philosophies and social systems." Although not aware of the real causes of the revolution Luria, in company with other young people, threw himself into the new movement because of the opportunities which it presented. In the opening chapter the liberation offered by the revolution in those early years comes across as a passionate, almost spiritual, excitement which served to fuel his intellectual endeavours.

Amidst the turmoil in Kazan University in the immediately post-revolutionary years Luria became absorbed with the then rather formless set of topics in the social and biological sciences which we now think of as psychology. After graduating in 1921 he began to investigate the effects of physical work on mental activity. His early studies on the role of verbal instructions on reaction time led to an enduring interest in the regulatory role of speech. He was also much interested at this time in psychoanalysis and had founded the Kazan Psychoanalytic Association.

In 1923 he moved to the Institute of Psychology in Moscow. The new Director, Kornilov, was engaged in eliminating all traces of subjective psychology and replacing it with objective methods — his "reactology". While appreciating the need for objective methods Luria avoided the sterility of this simple mechanistic scheme, his interest in psychoanalysis probably bolstered him in this. He designed a technique which he called the "combined motor method" to study the influence of emotions on voluntary behaviours. This early work, which was never published in Russian, shows clearly one of his trademarks — the application of objective methods to real life psychological problems.

In 1924 he met L.S. Vygotsky whom he describes quite simply as a genius. For ten years he worked closely with Vygotsky whose ideas plainly had a profound and enduring effect upon him. Vygotsky's 'cultural psychology' owed much to Marx's methods of analysis and to the intellectual climate released in Russia after the revolution. Inspired by Vygotsky's ideas Luria, Vygotsky and a small group of students set themselves the task of reconstructing psychology with man in his proper historical and cultural context. These ideas led Luria in the early 1930s to make two expeditions to Uzbekistan in Central Asia. Here a traditional peasant society profoundly influenced by a conservative Islamic culture was in the process of being transformed by collectivisation, literacy campaigns and formal education. The purposes and methods, along with an outline of the findings obtained from the two expeditions are described. This brief account of the work on cultural differences in thinking done almost 50 years ago is one of the most interesting chapters in an absorbing book. It deals with work which is little known in the Soviet Union and even less in the west. Following these pioneering studies Luria turned to work on twins in an effort to disentangle nature and nurture as they shaped psychological processes. From the account provided one cannot help but be struck by the originality displayed in the work and by the way in which Vygotsky's theoretical framework is woven throughout it.

Luria was perhaps best known as one of the founding fathers of neuropsychology. In the late 1930s he completed his medical training in Moscow and set about becoming, as he puts it, "a practical neurologist". He made notable contributions to our understanding of the aphasias, once again informed by Vygotsky's theory. When Russia entered the second world war in 1941 he was commissioned to organise a hospital for patients suffering brain injuries. The development of his ideas and methods of rehabilitation, especially in relation to speech, are simply and vividly described. The link between the war work and subsequent studies on the relationship between brain and behaviour, especially in connection with language function, is explored.

The final chapter in Luria's little memoire has the beguiling title, "Romantic Science". He distinguishes between classical science which is essentially reductive in nature, and romantic science which is concerned with the broader canvas of the whole person. In psychology the distinction and the corresponding dilemma have often been formulated in terms of the nomothetic versus idiographic approaches. Luria's work takes from both of these but in a period when the nomothetic approach is in the ascendency in western psychology his clear statement about the sources of ideas and about the importance of preserving the manifold richness of the subject, which he illustrates by his detailed studies of a man with an amazing memory and another man whose mind was shattered by a severe brain injury, provides a timely caution to us all.

In their introduction the editors provide a valuable historical context to Luria's psychological work. The final chapter is a sensitive and insightful essay by the editors in which they seek to give a picture of Luria the man. This is plainly difficult but the vicissitudes of his life are outlined and provide some explanation for the striking changes in direction which his work took, changes often brought about by political necessity. The two great influences on the development of Luria's psychological work were the revolution, which opened it, and Vygotsky whose ideas provided a connecting framework which ran throughout it. The atmosphere of the intellectual life which is revealed shows Luria to be a man possessed of boundless energy and insatiable curiosity — a great man. The book is quite simply a gem. Because of its simplicity and directness it reveals that most fascinating of things, another man's mind at work. ☐

Kevin Connolly is Professor of Psychology at the University of Sheffield, UK.

Objects of the Universe

David W. Hughes

Catalogue of the Universe. By Paul Murdin and David Allen, with photographs by David Malin. Pp.256. (Cambridge University Press: Cambridge, 1979.) £9.50.

cătálŏgue, n. a list or enumeration systematically arranged in alphabetical or other order, often with the addition of brief particulars.

ūn′ĭvêrse, n. The whole of created or existing things regarded collectively; all things, including the earth, the heavens, and all that is in them, considered as constituting a systematic whole.

ON reading these two dictionary entries the enormity of the task the authors had set themselves seemed overwhelming. Murdin, Allen and Malin have, however, assembled a much simplified catalogue of astronomical objects and planetary features. The book is a picture gallery of galaxies, stars, nebulae and planets, each photograph being accompanied, on average, by an equal area of prose. The balance is reasonable too: 26% galaxies, 36% stars and nebulae, and 38% planets.

In the preface the authors state that the photographs are the core of the book , and a "best-buy" policy has been adopted in selecting the ones for inclusion. They then bravely go on to say: "We hope that an astronomer browsing in the sections which lie outside his special field thinks that the best-buy choices are justified, even if he happens to disagree with us in the area he knows about." Well I did just that and obviously this makes me very worried. My field is the Solar System, especially the minor bodies in it — comets, asteroids, meteorites and meteoroids. The photographs in this section were poor — I'm sure I could have selected much better ones myself. Also having NASA slides in my collection of many of the Moon, Mercury and Mars photographs used in the book, underlined the fact that many of the reproductions in these sections are not of the best. However, as I wandered away from my field and browsed in other pastures I became more and more impressed. The Lagoon nebula looked sharper than I had ever seen it before, thanks to some of the special photographic techniques used for the illustrations. On the other hand, even here a common illustration like the Lund Observatory's composite photograph of the Milky Way has been so badly reproduced that much of the detail you know exists on the original is invisible, But I'm still worried about the author's admonition. May be if I meet an expert of that speciality in some distant pasture, wouldn't he be as disillusioned with his area as I was with mine? It's quite surprising isn't it how impressive certain books can be when they discuss things you haven't encountered before, only to become painfully weak when they turn to your own speciality.

The text also worries me. Common catalogues like stamp catalogues and horticultural catalogues at least tell you how big the stamps are, their perforation, the issue dates and print numbers; and when reading about the plants I expect to learn how tall and hardy they are or whether they like shade or sun. Common catalogues abound with quantitative details. Not so this one. Time and again I get the impression the reader is being talked down to. I want to know how rare, hot, massive, dense, luminous, old (etcetera, etcetera) these objects are, I'm not told often enough. If I've gone to the trouble of looking up "infrared radiation" in the glossary I want to be told more than that it is just heatwaves. Any author who writes that fireball trains are nearly as wide as the Full Moon sows seeds of doubt in my mind about his other prognostications.

The underlying, motivating, idea behind the book is extremely good. A catalogue of the objects of the Universe, well illustrated with a concise factual test would be most useful. Unfortunately with this book both the illustrations and the text let it down. Remove these and there is little to say other than that it has a pretty cover. ☐

David W. Hughes is Lecturer in the Department of Physics and Astronomy at the University of Sheffield, UK.

Physical optics

P.W. Hawkes

Optical Image Formation and Processing. By M. Françon. Pp. 213. (Academic: New York, San Francisco and London, 1979.) $19.50; £12.80.

THE past twenty years have seen a number of major developments in optics, but for the newcomer, their relative novelty simply means that older textbooks will not cover them and that some of the newer ones, or new editions of the old, will treat them as difficult advanced topics or in brief, all-too-often obscure appendices. Professor Françon is too experienced a teacher of optics to fall into either of these traps. He has written a short introductory text on physical optics, in which such topics as partial coherence, transfer functions, holography, speckle interferometry, image processing, lasers and optical waveguides are treated alongside and in exactly the same tone of voice as, for example, Babinet's principle and the Michelson interferometer. Students encountering physical optics for the first time will find the style clear and the mathematics simple and easy to follow. Only in one case did I find the clarity deceptive, namely, the treatment of speckle; here, I feel that the newcomer will find himself following the discussion effortlessly but without the least idea of what speckle is all about.

In a book intended for beginners, the quality of the translation is particularly important. The translator, B.M. Jaffé, has produced a text that is smooth and lucid for the most part, though here and there a technical phrase has eluded him. The only one that matters is "pattern recognition", for which B.M. Jaffé writes "form recognition" (from *reconnaissance des formes*). From personal experience, I know how easy it is to be hypnotised into translating into perfectly grammatical but somewhat unnatural English and B.M. Jaffé is occasionally, though not often, guilty of this: "If one agrees to allow the frequency to vary . . . " (p 59), for example, and " . . . it will be necessary to remind the reader of the basic properties . . . " (p 98). Here and there, I felt he might have inserted (if the author agreed) a helpful phrase; thus again on p 98 it would have been useful to mention that fig. 8.1 is commonly known as the Hurter-Driffield or H-D curve and to put "log W" in the text. On p 190, he really ought to have noticed that, although "The first of the two relations (A.28) is often given the name of Parseval's theorem", this is in fact wrong and Parseval's theorem states something different, which is easily derived from the relation in question.

Nevertheless, these are only very minor blemishes in a book that first or second-year undergraduates, revising for exams, will find immensely helpful particularly if Academic Press produce a paperback that they can afford.

P.W. Hawkes is Maître de Recherches in the Laboratoire d'Optique Electronique du CNRS, Toulouse, France.

Semiconductor physics

Andrew K. Jonscher

Semiconductor Physics. By P. S. Kireev. Pp. 693 (MIR Publishers/Central Books: London, 1977.) £5.95.

THIS is an interesting novelty — a translation from Russian into English made and published in the USSR and offered at the bargain price, by any standards, of less than six pounds for nearly 700 pages in hardback. The first point to make is that the translation is remarkably good coming from a non-native English speaker and it would be churlish to quibble over small points of odd usage of English. That aspect is quite satisfactory.

The material is organised into the following chapters (with numbers of pages): introduction (31), band theory (138), electron and hole statistics (54), kinetic phenomena (135), scattering theory (92), recombination (36), contact phenomena including p-n junctions (35), optical and photo-electric phenomena (101), Appendix — group theory (61). The size of the chapters reflects fairly closely the Author's predilections and the strong points of his expertise, which evidently centre on theory; all treatment of 'concrete devices' is excluded. The quantum mechanical treatment of the band theory of solids is very thorough — certainly at postgraduate level — and includes the effects of magnetic fields, surfaces and point defects, but not strongly disordered solids. Simple examples of band structure calculations are also included. Carrier statistics is treated conventionally, includes a lot of detail and covers, in particular the quantisation in high magnetic fields.

The treatment of transport begins with Boltzmann's transport equation — going into considerable detail with tensor effective masses — and includes the treatment of galvanomagnetic phenomena, especially the Hall effect, heat conductivity and thermoelectric effects. It is noteworthy that frequent examples are given of experimental data to illustrate particular developments in the theory. The principal carrier scattering mechanisms are described in considerable details, and a rather unusual feature of the book is that lattice vibrations and specific heats are dealt with as a sub-section of this chapter. There is a brief treatment of high-field effects and deviation from Ohm's law, but this general subject is treated in a rather disappointingly sketchy manner and manages to give a confusing picture of the Poole, Stark, Poole-Frenkel and Zener effects. The important topic of transferred electron phenomena receives barely one page and one not very informative diagram: — one would have expected more after the very thorough preparation in earlier chapters. There is nothing about effective carrier temperatures in high fields and band-to-band avalanche multiplication, and saturated drift velocity is shown in a diagram but is not discussed in any detail.

After the very detailed treatment of the band theory, the presentation of the physical aspects of carrier recombination is decidedly perfunctory and the subject of minority carrier injection does not receive any significant amount of attention, either at the level of analysis or of a physical description of ambipolar flow and preservation of neutrality. The chapter devoted to contacts includes a very sketchy description of the p-n junction but the voltage-current characteristics of neither of these are derived. This is perhaps taking the avowed aim of excluding devices a little far, as the I-V characteristic may be considered to be both fundamental to the theory of semiconductors and highly illuminating physically of a range of transport phenomena.

By contrast with the above, optical and photoelectric phenomena are treated again quite thoroughly, especially free carrier absorption, cyclotron resonance, plasma reflection, intrinsic absorption in considerable detail, lattice absorption and impurity absorption. Likewise, photoconductivity, photovoltaic effects and photoelectromagnetic effects are given reasonably good coverage, with illustrations relating to experimental results.

There is an appendix on group theory which is useful for the beginner, although the precise logic of including this rather than any other supporting material is not obvious. A good feature of the book are the brief summaries at the end of each section which should be useful for revision and for rapid scan of the principal features.

Although carefully selected passages of this text would be suitable for an undergraduate course, the treatment is definitely postgraduate, and rather specialised at that. The book is literally packed solid with information — the typographical layout is very condensed, with 46 lines to a page of slightly smaller format than an equivalent Western text with 38 — 39 lines. Similarly, mathematical expressions, of which there is a great deal, are tightly packed in the text and most of the diagrams are very small by our standards. Thus, although the reading process may be more tiring, the book contains a good 20% more information per page than its equivalent here.

The quality of paper is not high — especially as the book is liable to get thumbed readily in extensive usage. A very serious defect — not untypical of Soviet books — is the omission of an index, expecially in a text that is more likely to serve as a reference source rather than as a regular reading text.

Altogether, a very mixed impression. A first-class bargain in terms of the sheer quantity of information per pound sterling, good coverage of theoretical aspects such as band theory, transport and optical phenomena, but quality of treatment falling off rapidly in other areas. In those areas in which it is strong it should form a good reference source, although I would dread having either to teach or to read a course of that level of detail. Perhaps the publishers may be able gradually to take into account the different standards of presentation normally expected in the West, thus further increasing the attractiveness of the product, even if that meant raising the price by as much as 50% — it would still remain a bargain! ☐

A. K. Jonscher is Professor of Solid-State Electronics at Chelsea College, University of London, UK.

Yeast identification

B.H. Kirsop

A Guide to Identifying and Classifying Yeasts. By J.A. Barnett, R.W. Payne and D. Yarrow. Pp. 315. (Cambridge University Press: Cambridge, 1979.) £32.50.

THE yeasts are a heterogenous and important group of organisms which are widely distributed, commonly isolated and often in need of identification. This *Guide* has been written to help overcome difficulties in identification and is intended to be used in conjunction with the standard work *(The Yeasts,* edited by Lodder, North Holland, 1970). Those experienced in yeast identification will meet little difficulty and so the present work must be judged by the extent to which it helps the inexperienced.

The *Guide* provides condensed but useful descriptions of methodology and of the principles of clasification. It lists the salient properties of each genus and provides a particularly valuable tabulation of the major physiological and morphological attributes characteristic of each species. Identification is based on keys, a general one being provided for yeasts as a whole; there are also special keys to smaller groups, such as those likely to occur in particular habitats or those possessing specific morphological or physiological attributes. Testing with unnamed yeast showed that the keys are easy to use and readily yield names. In a significant proportion of cases this name differed from that deemed appropriate when all the properties of the organism were considered; the discrepancies arose either because an organism was atypical for its group in a characteristic used in the key — all microbiologists know well the atypical organism — or because of difficulty in interpreting test results. The authors recognise the problem, for they provide a useful summary of the features which distinguish each species from all, or all but a named few, other species; and this helps the inexperienced to detect errors.

In spite of this drawback, the *Guide* provides much useful information which is particularly valuable for the expert, for it brings together in one place much information published since the 1970 edition of *The Yeasts*. Since then many new species have been described and massive reorganisations of taxonomy have been recommended, accepted by the authors and incorporated into the keys. Although this makes it difficult to use the *Guide* in conjunction with the 1970 edition, as recommended by the authors, the ready accessibility of this information is nevertheless most welcome. One is tempted to wish that the authors had produced a still more substantial monograph which would have been of great value to the beginner and to the expert alike. □

B.H. Kirsop is Head of the Microbiology Division at the ARC Food Research Institute, Norwich, UK.

Dealing with information in the nervous system

H. B. Barlow

Information Transmission in the Nervous System. By A. M. Uttley. Pp. 111. (Academic: London and New York, 1979.) £7.50; $15.50.

It is obvious that Pavlov's dogs, who salivated when a bell sounded, had in some way formed an assessment of the conditional probability of food, given the sound of a bell; because the dogs' past history had been controlled so that this probability was high, they salivated on the sound of a bell, without waiting for the food. In this sense, the nervous system must be able to compute conditional probabilities, and this is the starting point for the work, stretching back over 30 years, that Albert Uttley summarises in this book.

I think he is right in giving central importance to the question how conditional probabilities are assessed in the nervous system, and I also like another feature of his approach. Given its central importance, he says, let us explore the hypothesis that modifiable synapses in the nervous system are commonly arranged in such a way that the modified synapse stores a conditional probability. The book is a development of this theme, and centres on the "informon", an element for which a stored variable, Y_i, representing the effectiveness of a synapse, is given by

$$Y_i = -k\log(P(X_i \text{ and } Y)/(P(X_i) \times P(Y)))$$

where k is a positive constant, and $_i$ and Y are two binary signals, the X's usually representing inputs and Y an output. Y_i is thus the negative of Shannon's Mutual Information Function for X_i and Y. Before going into further details two warning are required.

First, the title might mislead the unwary reader. It is not a general treatise on how information, in the general sense, is transmitted in the nervous system. Rather, the title was chosen, I suspect, to emphasise that "informons" adjust themselves in accordance with "information" in the specialised Shannon sense, rather than just messages; they store the conditional probabilities that enable them to deal effectively with information.

The second warning is that, although I am sympathetic to the author's general purpose and train of though, I did not find the book easy to read and follow. The author has perhaps lived with the ideas so long that he no longer has the perspective necessary in order to explain them effectively to newcomers.

The book contains eleven short chapters. The first four explain the concepts and illustrate how a theoretical "informon" might be realised by a nerve cell with modifiable and unmodifiable synaptic imputs. The next two chapters match the conceptual informon's behaviour against that of granule cells in the hippococampus, as shown by the work of Bliss and Lomo in rabbit and Douglas and Goddard in rat. He then deals with classical conditioning, the use of informons for pattern recognition, the interpretation of plasticity in the visual cortex, and the extension of the ideas to operant conditioning.

The book is a valiant and rather isolated effort in an original direction. It should be read by those who seek an explanation for quite complex psychological facts in elementary properties of synapses and nerve cells, and it thus forms a contrast with explanations for those same facts in terms of "programs" that could be realised by any kind of computing machinery. Conditional probabilities and Shannon's Information are quantities that form conceptual bridges between the two approaches, and the most valuable feature of Uttley's work may be to show how much can be achieved directly by neural hardware adapted to computing these quantities □

H. B. Barlow is Professor in the Physiological Laboratory, University of Cambridge, UK.

Objectives of agricultural policy

R. F. Lord

Government and Agriculture: A Spatial Perspective. By I. Bowler. Pp.127. (Longman: Harlow, 1979.) Paperback £4.50.

Dr Bowler is one of a growing band of geographers concerned not only to describe, but also to hypothesise. In this book he takes the view that "a spatial perspective can provide additional insights into the government — agriculture relationship". That he only partially succeeds in his task is due largely to the fact that objectives of agricultural policy rarely contain a specifically spatial dimension and that even where they do, statistically acceptable data are seldom available on a spatial basis in order to measure achievement.

The first part of the book drawing on experience in a number of developed countries, with a wealth (surfeit?) of references, considers how agricultural policy has been influenced by the changing nature of the "agricultural problem" and concludes that for the most part there has been a failure to achieve the policy goals so optimistically set by governments.

The second part of the book relates solely to the development of UK agricultural policy in the post-war period up to 1973. Only in the last two chapters (6 and 7) is attention devoted to the spatial aspects of the problem (which considering the title of the book is surprising). Generally the ineffectiveness of measures in a spatial sense is demonstrated. However, Dr Bowler's concern for the spatial dimension is justified in his concluding paragraph where he refers to the need for more research of this type if problems of formulating adequate regional policies in the EEC are to be overcome. □

R. F. Lord is Head of the Economics and Management Department in the School of Agriculture, University of Edinburgh, UK.

nature

28 February 1980

Smoking and health: time for a legal commitment

THE extent to which Mrs Thatcher's government is prepared to discourage smoking will become clear within the next few weeks. For by the end of March the UK government and the tobacco industry are due to have renewed their voluntary agreement on advertising. And on 26 March the tax on tobacco can be adjusted in the budget. It will be a great pity if either opportunity to discourage smoking is wasted.

Given that one in ten UK deaths can be directly attributed to smoking and that one smoker in four dies10-15 years prematurely, there can be no question about whether smoking should be discouraged. The question is:how? There are three traditional answers. The first is to increase the price of tobacco, the second is to reduce advertising and the third is to educate the public on the dangers of smoking.

On the issue of price, the UK National Consumer Council has recently produced figures which show that, in real terms, the retail price of cigarettes in 1978 was only 76% of what it had been 10 years before. Given that price increases are commonly held to be the most effective and lasting way of reducing cigarette consumption, there is a very strong case at least for restoring the relative cost of smoking by increasing the tax on tobacco in the next budget.

The advertising of cigarettes is already limited by a voluntary agreement that was negotiated between government and industry in 1977. This stopped the advertising of cigarettes on television or during cinema performances to which the young were admitted. Other forms of advertising were not stopped but controls were placed on the image of smoking portrayed. It is difficult to prove that the level of advertising has any effect on the consumption of cigarettes rather than, as the industry argues, just on the choice of brands — but it seems prudent to assume it does. The immediate aim of the government should therefore be to limit advertising still further and ultimately to the point of sale. Furthermore, at the end of the year, the government should make the most of its opportunity to renegotiate the voluntary agreement on sponsorship of sport by the tobacco industry, a promotional exercise to the tune of an annual £10 million that has done much to associate smoking with health and youth in a way that advertising has not been allowed to do since 1977.

As for anti-smoking campaigns, on which the government spends something like one hundredth of the industry's budget for promotion, the cheapest and most direct way of publicising the hazards of smoking is on the cigarette packages themselves. There is much scope for expanding and varying the present trite warning that "cigarettes can seriously damage your health".

Any attempt to reduce the consumption of cigarettes, however,needs to be complemented by efforts to reduce the risks to those who continue to smoke regularly. This currently includes 45% of male and 37% of female adults in the UK; the prevalence is falling but only markedly so in the higher socio-economic classes — for example among professional males the prevalence of smoking has fallen from 33% in 1972 to 25% now, whereas 60% of unskilled male manual workers still smoke compared with 64% in 1972.

One erstwhile promising approach to reducing the health hazards of smoking has clearly come to nothing. The idea was to replace some of the tobacco in cigarettes by a substitute that was, at best, harmless. A great deal of money was invested by the tobacco industry in the development of two tobacco substitutes. Early in 1977 the first report of the Independent Scientific Committee on Smoking and Health (the Hunter Committee) concluded that neither substitute was likely to be more harmful to health than tobacco alone and cigarettes containing them were launched in the UK later that year. But, as the recently published second report of the Hunter Committee says, sales of these cigarettes only briefly accounted for more than 2% of total sales.

Much greater success has met the introduction of low-tar brands of cigarettes and their acceptance by young smokers is probably the major cause of the declining trend in lung cancer deaths in UK males of less than 65 years of age. While welcoming the declining tar yields and recommending further substantial reductions, the second report of the Hunter Committee is less than forceful in its demands. It is left to the minority report of Dr J. D. Ball to point out that the average tar yield per cigarette sold in the UK has fallen very little since dropping 40% between 1965 and 1973. What is more disturbing is that the committee so strongly emphasises that reductions in tar yield will be limited by consumer acceptability of low-tar cigarettes. That appears to be a viewpoint that is more concerned with protecting the industry than it is with testing the limits of acceptability as a matter of urgency. Meanwhile the tobacco industry is apparently eager to spend £1 million on epidemiological studies of those who switch to low tar cigarettes rather than to admit that there is a case for reducing the tar levels now.

The attack on smoking can be pursued down a variety of paths, each one of which deserves to be explored vigorously. Voluntary agreements between government and industry are to be welcomed if they work — but there is considerable evidence that the tobacco industry has been less than scrupulous in honouring its commitments. That being so, the government should not be afraid to replace voluntary restrictions with legal ones if the tobacco industry is reluctant to agree to much stiffer controls on its distasteful business. □

0028-0836/80/09801-01$01.00

Environmentalists warn of continued threats to water supplies

DESPITE progress in reducing the more obvious sources of water pollution, US regulators are beginning to feel that problems in maintaining a clean and healthy water supply will play as controversial a role on the political agenda of the 1980s as the energy crises has done during the 1970s.

The latest warning of difficulties still to be faced comes from the Council on Environmental Quality, the watchdog committee established by President Nixon in 1970. In its latest annual report, published in Washington last week, the council says that the general standard of air quality has increased substantially over the past decade. In contrast progress in cleaning up water supplies has been far less impressive.

In particular, the council says that ground-water contamination from various activities, such as the use of pesticides in agriculture or the improper disposal of toxic wastes, is becoming a major problem for drinking-water supplies in parts of the country.

It draws attention to the impact of "acid rain" on wildlife and water quality: and looking ahead, picks out the problems of carcinogens in drinking water, supplies to "water-starved areas", and ageing distribution systems as three "basic and difficult problems" that the nation will have to face in the next few years.

Coming to grips with such problems may require new legislation and substantial resources: updating distribution systems, for example, could cost $50 billion to $100 billion nationwide to prevent large-scale disruption of supplies.

Yet left unsaid by the CEQ is that the Environmental Protection Agency is already struggling to carry out existing responsibilities which it has been given by Congress and in many cases the agency is meeting strong opposition from water utilities, who claim that new regulations to control toxic substances in water, for example, are over-restrictive and unnecessarily expensive.

Not that the picture is all gloom. The CEQ's report points out that there has been considerable success in controlling efficient emissions from factories and other identifiable industrial activities since the Clean Water Act was passed by Congress in 1973.

But statistics illustrate the severity of the problems that remain. Thus the report says that as many as two-thirds of the nation's lakes may have serious pollution problems and that an estimated 80% of the 37,700 lakes in urban areas in the US are "significantly degraded".

This pollution is inevitably taking its toll of wild-life. In many lakes and rivers, toxic chemical contaminants such as PCBs have made fish inedible. Acid rain has made many lakes, particularly in the north-eastern United States, lethal to fish and other aquatic life and four million acres of commercial shell-fish waters have been closed.

The extent of the continuing pollution problem is also demonstrated by the fact that there has been "little or no overall change in the levels of five major water pollution indicators — fecal coliform bacteria, dissolved oxygen, total phosphorus content, total mercury, and total lead — over the four years 1975 to 1978, the CEQ reports.

Industry, in some ways a comparatively easy target for the EPA, has responded relatively well to control legislation. Although some industries, in particular the steel industry, have resisted stringent effluent restrictions, 90% of industrial discharges now meet "best practicable technology" standards laid down in 1977.

Municipalities responsible for water treatment have been more of a problem. Although federal funds have been made available for sewage treatment facilities since 1973, for example, many urban areas have been reluctant to take them up. Elsewhere construction projects have been plagued by cost-overruns and profiteering.

As a result, municipal sewage wastes are still "years away from being totally controlled" says the CEQ.

Furthermore both industry and water utilities are responding warily to what one EPA official refers to as the "second generation of water clean-up" shifting attention from more well-known and obvious pollutants to the long term effects of relatively low levels of toxic substances.

The agency, for example, is already experiencing considerable difficulty in carrying out some of the requirements of the Safe Drinking Water Act of 1974, introduced after environmentalists found abnormally high cancer rates among residents of Louisiana who used water supplies from the Mississippi river.

Further difficulties are likely to face the EPA as it moves to implement industry-by-industry guidelines for the regulation of 129 top priority pollutants — mainly heavy metals and substances known or suspected to be carcinogenic — agreed after pressure from groups such as the Environmental Defense Fund to follow through on the agency's congressional mandate.

Then there is the growing problem of acid rain. This is caused when emission from automobiles or power-plants combines with water in the atmosphere.

Here the CEQ points out that in the eastern half of the US the acidity of rainfall appears to have increased about 50-fold during the past 25 years. In addition to eliminating fish from lakes in the north-eastern US and Canada, acid rain, it says, "is also suspected of contributing to the leaching of toxic compounds into drinking water, especially surface water supplies".

All these problems are likely to mean increased responsibilities for the EPA. And the CEQ indicates that it is well aware of the difficulties faced, for example, in shifting the focus of control from point to "non-point" sources of pollution, such as run-off from construction sites, agricultural land or chemical dumps.

On top of all the other problems, the EPA is now faced with a bill introduced into the House of Representatives by Representative Phill Gramm of Texas, which would seriously limit its ability to enforce new quality controls: few believe the bill would pass solely on its technical merits but it could ride on the back of popular anti-regulatory sentiment and win a surprise victory. **David Dickson**

Panel to assess formaldehyde health effects

THE US Consumer Product Safety Commission announced last week that it is to set up a panel to help assess the human implications of research findings that exposure to formaldehyde can cause cancer in laboratory rats. The panel will be established in conjuction with the National Toxicology Programme, and will be assembled from various federal health and safety agencies and scientific organisations.

Last October, scientists from the Chemical Industry Institute of Toxicology and the Formaldehyde Institute reported that three laboratory rats of several hundred exposed to formaldehyde gas had developed prominent nasal cancers which were identified as squamos cell carcinoma.

Health scientists from CPSC and other regulatory agencies were told last month that such cancers were now evident in 37 of the rats in the study, which is three-quarters of the way through its two-year duration. In a random sample of rats selected for sacrifice and pathological examination after exposure at the dose level of 15 parts per million, 20% showed evidence of squamous cell carcinoma. □

0028-0836/80/09802-01$01.00

Scientists protest at exclusion of Russians from conferences

US scientists reacted with anger and embarrassment last week to the decision by federal authorities to bar Soviet-bloc delegates from two scientific conferences, one on computer technology the other a joint conference on laser engineering and inertial confinement fusion.

The decision appeared to contradict earlier statements by the administration that, although cutting off high level exchanges with the USSR following the invasion of Afghanistan and the exile of Andrei Sakharov, it intended to maintain lower-level scientific links.

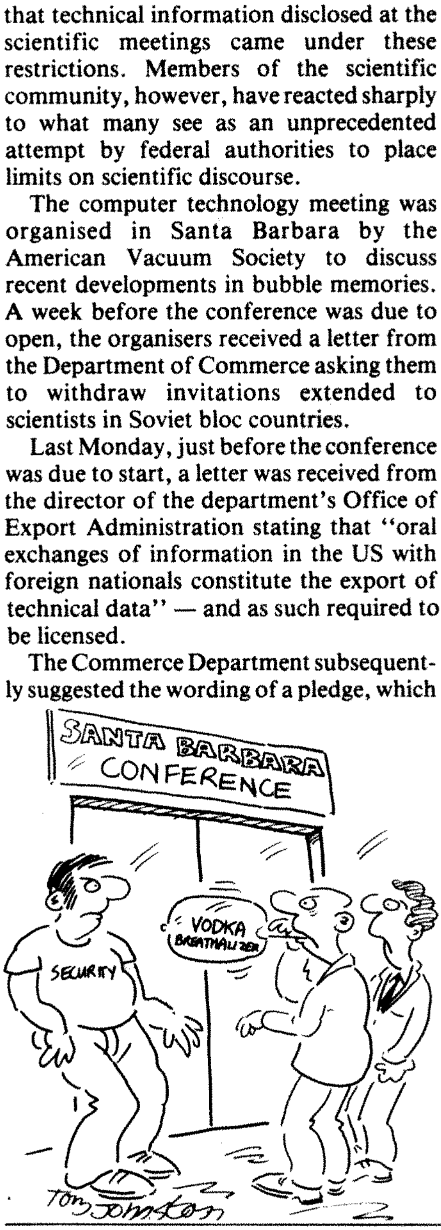
But administration officials point out that the decision to exclude Soviet participation was taken to restrict high technology exports.

Commerce and State Department officials, acting apparently without prior consultation with the White House, argued that technical information disclosed at the scientific meetings came under these restrictions. Members of the scientific community, however, have reacted sharply to what many see as an unprecedented attempt by federal authorities to place limits on scientific discourse.

The computer technology meeting was organised in Santa Barbara by the American Vacuum Society to discuss recent developments in bubble memories. A week before the conference was due to open, the organisers received a letter from the Department of Commerce asking them to withdraw invitations extended to scientists in Soviet bloc countries.

Last Monday, just before the conference was due to start, a letter was received from the director of the department's Office of Export Administration stating that "oral exchanges of information in the US with foreign nationals constitute the export of technical data" — and as such required to be licensed.

The Commerce Department subsequently suggested the wording of a pledge, which all foreigners attending the conference were obliged to sign, stating that they would not reveal any information obtained during the meeting to citizens of the Soviet bloc or other communist countries — including China.



(Three Chinese scientists who had arrived in Santa Barbara for the meeting were also excluded but were admitted on the second day partly, it is believed, as a result of the intervention of the Office of Science and Technology Policy which has been actively engaged in establishing US-Chinese links in science and technology).

The meetings on laser engineering and inertial confinement fusion are being held this week in San Diego, and are jointly organised by the Institute of Electrical and Electronic Engineers and the Optical Society of America.

A Russian post-doctoral student currently studying at the University of Texas, who had had a paper accepted for presentation at the meeting, was subsequently refused permission by the State Department to travel to San Diego to deliver it. On enquiring about this decision, Dr Jarus Quinn, President of the Optical Society, says the State Department told him that several Russian scientists had applied for visas to attend the conference, but that these would be refused.

Commerce Department officials insist that they are merely carrying out the intent of legislation restricting Soviet access to US technology and that the pledges required of participants in the Santa Barbara meeting were necessary to avoid violating technology transfer laws. But the scientists see it differently.

Dr J. Allen Bromley, professor of physics at Yale University and a past chairman of the International Union of Pure and Applied Physics, said that the US action might infringe a position statement issued by the International Committee of Scientific Unions on the behaviour expected of a country hosting international scientific conferences. "If allowed to stand it could damage in a very serious way US participation in international science," he said.

Mr William Carey, Executive Officer in the American Association for the Advancement of Science, commented that the government's actions reflected a "very summary procedure", to interfere so awkwardly in a non-governmental scientific conference.

"It is one situation where the government is managing exchanges carried out under agreements made with another government. It is quite another to interpret a statute and invoke it with no precedent to limit scientific discourse that has been called under private auspices", he said. ☐

Carter terminates work on nuclear waste disposal project

PRESIDENT Carter has announced the cancellation of the Waste Isolation Pilot Project (WIPP), planned to study the result of depositing high-level nuclear wastes in salt mines near Carlsbad, New Mexico. He argued that to proceed would be inconsistent with the administration's strategy of comparing sites with differing geological characteristics before agreeing to the construction of test disposal facilities.

The President's decision was announced as part of a comprehensive radioactive waste management programme, in which decisions to initiate construction of waste disposal plants are to be put off until further knowledge has been obtained on the behaviour of mined geological repositories capable of accepting waste from both reprocessed and unreprocessed commercial spent fuel.

Final decisions on many steps which need to be taken "should be preceded by a full environmental review under the National Environmental Policy Act", the President said. He added that the Department of Energy had already mounted an expanded and diversified programme of geological investigation that recognised the importance of the interaction among geological setting, repository host rock, waste form and other engineered barriers on a site-specific basis.

"Immediate attention will focus on research and development, and on locating and characterising a number of potential repository sites in a variety of different geological environments with diverse rock types", the President said. "When four or five sites have been evaluated and found potentially suitable, one or more will be selected for further development as a licensed full scale repository."

President Carter's decision to terminate the WIPP project, long opposed by environmentalist groups in New Mexico, was also partly based on his desire that all repositories should be licensed. The involvement of civilian licensing bodies had been opposed by defense officials, who would also have used the facility for storing high-level wastes.

As part of his waste storage programme, the President also announced the establishment of a state planning council of elected state, local and tribal officials and heads of cabinet departments and other federal agencies. "The basis of the relationship between states and the federal government in the siting of high level waste repositories will be the principle of consultation and concurrence," the President said. ☐

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United Kingdom

PWRs unlikely to be safe, says metallurgist

CRACKS in the pressure vesels of pressurised water nuclear reactors would be very difficult to detect before they led to catastrophic failure, Sir Alan Cottrell, ex head of the University of Cambridge Department of Metallurgy, told the House of Commons Select Committee on Energy last week. Britain should therefore reverse its decision to build a series of PWRs, and adopt the advanced gas-cooled reactor instead, he argued.

Sir Alan appeared undismayed by the delays in the UK's AGR programme. "They are constructional" he told *Nature*. "The Hinkley Point AGR has performed extremely well." (Hinckley Point, the only AGR in operation, achieved 80% of its design capability in 1979, with an output of over 1000 MW, the Central Electricity Generating Board said this week.)

Sir Alan's first objection to the PWR was that it uses water rather than carbon dioxide as a coolant. "There is a natural safety in CO_2 which H_2O lacks" he says in his written evidence. This is that CO_2 remains gaseous under all reactor conditions, whereas H_2O can exist in two phases — liquid and gaseous — "with very different densities, coolant properties, heat contents, and pressure-volume-temperature relationships".

This leads to two safety disadvantages. First, a PWR must be operated at 150 atmospheres pressure to keep the H_2O liquid at its operating temperature of 320°C; and second "the problems of controlling an irregularity in the operation of a reactor are exacerbated if there is a risk of the coolant suddenly changing its physical state and losing its coolant properties".

The problems which faced the operators at Three Mile Island — "the speed of changes in the reactor, the false indications of the physical state of the coolant, the rapid overheating of the fuel due to the disappearance of the water" — would not have arisen in an AGR "with its lower power density, large mass of heat-absorbing graphite and invariably gaseous CO_2 coolant".

The second objection Sir Alan raised was that it would not be possible to detect a crack in the pressure vessel by the leaking of radioactive material until it was too late.

"To hold the water pressure, the walls of a PWR vessel and circuit have to be thick, up to 14 ins in places, and are made of a fairly hard, strong steel. The thickness and hardness deny the possibility of 'leak before break'. For under these conditions, the critical crack size at which a sharp crack will spread rapidly and uncontrollably is expected normally to be about four inches but could be as little as one inch under fault conditions. It follows that, if a wall becomes perforated by a growing crack, while under operational pressure, the crack is already unstoppable and will spread to form a major break within a millisecond."

"The security of the reactor thus depends on the avoidance of critically-sized cracks or similar defects in the steel."

Dr Walter Marshall, deputy chairman of the United Kingdom Atomic Energy Authority, had chaired a study of pressure vessel integrity and concluded, according to Sir Alan, that "the assurance of initial safety depends on meeting a number of exacting conditions about standards in workmanship, care in operational control, and rigour in inspection". These conditions, said Sir Alan, "call for considerable human abilities". It was a matter for general judgement "whether this degree of reliance on human abilities provides an adequately sound basis for the safety of a nuclear reactor".

Another worry, said Sir Alan, concerned the possible growth of small cracks, harmless at first, due to metal fatigue and corrosion. If these were discovered (as they have been in some French PWRs) there would remain the problem of repair in reactor conditions.

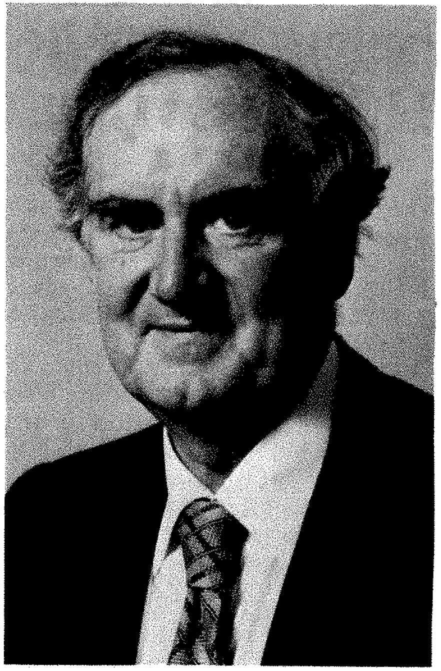
"The task of developing new equipment to grind out and soundly weld up cracked regions in radioactive steel, remotely by automatic methods, is a formidable one. But if such cracks were allowed to remain and were then to grow large, a government would be faced with a most difficult decision: either to take the chance of running the reactor . . . or to shut-down the reactor at a fraction of its planned economic life."

"The long-term value of PWRs may thus depend on the development of a new technology for the remote repairing of thick radioactive steel."

Dr Walter Marshall said on Monday that he accepted the technical basis of Sir Alan's arguments as "correct and completely valid". But "by selecting facts you can give the wrong impression".

For example, on the positive side of water as a coolant is that it is also a moderator — so loss of coolant will also stop the nuclear reaction.

On the "considerable human abilities" required of pressure vessel manufacturers, Dr Marshall said "there are firms abroad that can attain the necessary standards. For example, we could get steel from Japan; and among others Framatome in France and GHH in Dusseldorf could fabricate the vessels."



Sir Alan Cottrell: doubts about pressure vessel integrity

Was it wise to rely on Framatome, which has discovered cracks in pressure vessels it has built for French reactors? "Well, their big advantage is that they are busy. They may have made one boo-boo but they've learned from it."

"The technology is moving to larger and larger forgings" said Marshall "which you weld together. So ultimately it's the steel manufacturer who matters most. In England River Don in Sheffield could do it."

Dr Marshall does not want to be "sloppy" about inspection. "I want to be able to lay my hand on my heart and say, that one's OK." He would like to have "the full resources" of the UKAEA behind him; the authority itself should be the inspectorate.

If cracks are found, they must be repaired *in situ*, a task which will not be impossible, thinks Marshall. "You would grind out the crack, and not bother to replace the cladding. The water would just get a little rusty." Six specially protected divers successfully replaced the complete reactor support structure under water inside the 'Stada' German reactor in 1973, said Marshall.

Monitoring known cracks, like Framatome's, would not be difficult, but "the most sophisticated, the fundamental question is whether your initial inspection misses any cracks. I couldn't emphasise the importance of the initial inspection too much" said Marshall.

Commenting on Dr Marshall's remarks Dr Cottrell told *Nature* that he feels Dr Marshall has underestimated the repair problems but "we agree on most technical questions; we differ on where we go from there. I take a less cheerful view on whether this rigorous inspection can be achieved. In my view, it's simply asking too much."

Robert Walgate

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Hamburg Scientific Forum

No agreement yet over minimising bureaucratic barriers to exchange

INTERNATIONAL scientific exchange must not merely take place; it must be seen publicly to take place. That is how one delegate to the Hamburg Scientific Forum has explained its purpose. The Forum has brought together leading scientists from Europe and North America for two weeks in the service of what has been referred to as the CSCE process, i.e. the implementation of the Helsinki Accords on peace and security in Europe.

The Soviet invasion of Afghanistan and the internal exiling of Academician Andrei Sakharov to Gor'kii mean that the forum is convening among what Dr Armand Boever of Luxembourg calls the "menacing strom cloud of the last few weeks". Inevitably the opening statements by the participating countries delivered last week, put considerable emphasis on the concepts of academic freedom and the responsibility of scientists towards society.

Strong statements on the human rights issue, sometimes citing the Soviet Union by name, sometimes, as did Dr Andre Lwoff (France), referring to breaches of the Helsinki accords in "a certain country", drew an emphatic refutal from the leader of the Soviet delegation, Dr Nikolai Blokhin. "The basic objection is that we observe our laws", he protested, speaking under the right to reply. "There have been no violations of Soviet law in the Sakharov affair. And Gor'kii is not exile. I myself was born there."

This undertone of confrontation which ran through the entire two and a half days of opening statements, focused attention on the fact that the forum was not, properly speaking, a scientific meeting. When, finally, the preliminary statements were over, and the meeting divided up into working groups on pre-selected topics (alternative energy sources, food production, cardiovascular, tumour and virus diseases, human environment and urban development), the scientists in the various delegations, irrespective of their countries' political system, seemed only too anxious to come to grips with the basic problem of promoting international scientific exchanges.

The majority of western delegates felt that if the forum were to make any real contribution to international exchange — as opposed to simply demonstrating scientific cooperation in the service of detente — it could best do so by endeavouring to eliminate or at least minimise the bureaucratic barriers to such exchange.

Since almost all the 35 delegations contained a sprinkling of bureaucrats, who sat in on the various working groups, the forum seemed an ideal opportunity for the scientists to make known their grievances against official frustration and obfuscation. This, however, various Soviet delegates were unwilling to allow. Repeated attempts to raise, in the closed working sessions, questions of visas, the cancellation of visits at the last moment for no clear reason, the substitution at conferences of some scientific nonentity for the speaker originally invited, and all the other difficulties and disappointments familiar to the would-be hosts of Soviet scientists, were quashed by the various Soviet representatives. "We are not here to discuss such matters" was their attitude — in which case, one might well ask with Dr Th. P. Tassios (Greece) what unique purpose the forum could serve.

Dr Tassios suggested that a permanent steering committee should be established to "define and shape" scientific "goals" and to deal with the whole problem of the ethics of science. To judge from the opening statements and the general tone of the Forum so far, this is a minority view. Most delegates, at least from outside the socialist bloc, seem to agree with Lord Todd (UK) that whatever the forms of future cooperation to be agreed by the Forum, more organisations to take scientists away from the bench and into the conference jet-set are not needed.

Vera Rich

United Kingdom

Split over admissible evidence at test drilling inquiry

THE UK's first public inquiry into test drilling for the possible disposal of high level radioactive waste got off to a stormy start in Ayr, Scotland last week, with claims that the government was trying to cover up the real issues by restricting the type of evidence. The Scottish office has ruled that it will accept testimony only on the limited question of test site drilling. Community groups, fearing that limited approval for site drilling will create a precedent that will prejudice further hearings, have argued that evidence on the question of the suitability of the area for waste disposal itself ought to be admitted.

The inquiry had been set up to consider a planning application lodged by the United Kingdom Atomic Energy Authority two years ago to test drill an area of hard granite above Loch Doon in the Galloway Hills — possible sites in the UK for Britain's first underground radioactive waste repository. Soon after the planning application was lodged the local authority, Kyle and Carrick District Council, turned it down. The inquiry that opened in Ayr last week is the result of the UKAEA appealing against that decision.

The first witness, Scottish Under Secretary William Scott, stressed that the government had not yet decided on the best way of disposing of nuclear waste. Research was also planned on waste disposal on and under the sea bed, he said. Under cross examination Scott admitted that he could give no firm undertaking that all the other 15 shortlisted sites would be fully investigated at a cost of £1 million each.

Byron Lintern, a senior scientific officer from the Institute of Geological Sciences, and a witness for the UKAEA, explained that the proposed test drilling would involve sinking approximately 40 boreholes over a wide area at varying depths down to a maximum of 500m. Two 15m rigs would be needed and a staff of about 15. Drilling would be phased over a two or three year period to avoid disturbing local birds during their breeding cycle. The cost of test drilling had risen from £150,000 in 1978 to £450,000 in 1980, he said.

Dr Lewis Roberts for the UKAEA told the inquiry that the UK currently had about 1,000 m^3 of high level waste stored in steel tanks at Windscale. It was proposed to glassify the waste, encase it in non-corrosive metal and then find the most suitable place to store it. A safe home had to be provided for around 500 years, by which time gamma radiation would have decayed to acceptable levels. Roberts rejected Kyle and Carrick's suggestion that the drilling work was "premature."

● ONE of the main objectors at the formal inquiry, the Scottish Conservation Society, organised its own parallel hearing — the People's Planning Inquiry Commission — to discuss nuclear waste dumping, safety and alternative methods of disposal, topics forbidden at the main inquiry. 99% of those attending the 'People's Inquiry' were opposed to the drilling proposals.

The UKAEA declined an invitation to attend, but paid the expenses of Professor John Fremlin, a radiation biologist, who told the meeting that there was little likelihood of the proposed dump causing serious contamination. It would take 100,000 years for the waste to find its way to the surface, he said, and in the worst possible situation would only then result in one death per decade.

Ted Stevens

Ted Stevens is a freelance journalist specialising in planning

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The Baltic

States cooperate to check sea pollution

WITHIN the next few days, West Germany, the last of the seven signatories to the Helsinki Convention on the Protection of the Marine Environment of the Baltic Sea area, is expected to deposit its instrument of ratification with the Finnish government. Two months from that day, the convention will come legally into force — the only such agreement to date to cover every aspect of the protection of the marine environment. The delay, caused by federal structure, has not, however, prevented the contracting countries from already embarking on a major programme of work on Baltic ecology.

The Baltic water is of exceptionally low salinity. This results in an ecology of low diversity, which is extremely vulnerable to environmental change. Accordingly, no sooner was the convention drafted in 1974, than a working party, the Interim Commission, was set up to carry out a number of preliminary tasks, including getting to grips with the scientific problems.

Foremost among such tasks is the permanent monitoring of the Baltic for pollution. The convention defines potential pollutants as hazardous (DDT and the PCB and PCT compounds), noxious (heavy metals, radioactive substances, etc) and oil. Seven of these have been selected as immediate priority areas (till 1983), with responsibility for research assigned on the lead country principle: Denmark for PCB and PCT's, West Germany for lead, East Germany for copper and zinc, Poland for DDT, USSR for mercury, Finland for oil, and Sweden for cadmium.

Basic research is not directly the concern of the Commission, although it does serve as a coordinator of the results of the environmental programmes of the individual countries. (Its cumulative bibliography of relevant publications in the Baltic countries since 1970 will be published shortly and there is a proposal to set up an international Baltic library). The main task of the Commission is to work out a coordinated monitoring programme, permitting the direct comparison of results.

A five-year pilot programme was initiated in March 1979. Sampling is to be carried out both at coastal stations and at specified locations at sea. Tests include both the physical monitoring of sea water parameters, and also biological specimens — notably herring, cod, *Macoma baltica,* eider duck, arctic tern and red breasted merganser. To ensure comparability, especially with the biological specimens, a massive file of methodological guidelines, covering all aspects from age and maturity of the specimens selected to test procedures and presentation of results, has been compiled. This is not yet in its final form, but, according to Dr Ilppo Kaugas, the scientific expert of the Interim Commission, only a very few minor comments and amendments have been proposed.

Intercalibration workshops form an important part of this monitoring programme. To date, these have included a chemical intercalibration workshop at Kiel in 1975 and one on biological calibration methods in 1979. A meeting on biomass, financed by the Commission and organised by Polish marine biologists, will be held in Gdynia in May of this year. In general, Dr Kaugas told *Nature*, the methodology for secondary production is still somewhat vague, and much work may be expected in this field in the future.

The most urgent problem, however, is that of oil spills and ship safety. In the narrow confines of the Baltic, even a close in-shore spill can affect the coasts and waters of other countries. The February 1979 spill of Ventspils harbour (Latvian SSR) ended up on the shores of Sweden and Finland. One recent idea, put forward by a Swedish team, is that all oil cargoes should be "tagged" by the addition of a mixture of metal particles. In the case of a spill, the relative composition of the tagging mixture could be determined by microscopic and mass-spectrographic methods, and this composition, which would be specific to each ship, would, when the convention comes into force, be considered as legal evidence, comparable to a fingerprint.

A large-scale international experiment on tagging methods was mounted last autumn, investigating such factors as the effect of climatic and meteorological factors on tagged oil. According to Dr Kaugas, the experiments were, in principle, a success, and similar methods "or possibly better" may well be introduced in the future.

Much attention is also being paid to general problems of ship-safety, including the logistics of long-distance pilotage of incoming tankers from the Kattegat to their home ports. One major problem facing the Commission is, however, that of ships sailing under the flags of countries not covered by the convention. "Everything depends in this case on the goodwill of the masters sailing under foreign flags", Dr Kaugas explained. For, although it was at the UN Conference on the Human Environment in Stockholm in 1972 that the Finnish delegation first raised the necessity of international action on the Baltic, and although it is proposed that, when the Permanent Commission is in operation, it will supply data from its monitoring programme to the UN Regional Seas Project, the Helsinki Convention is, essentially, a private agreement between the seven countries bordering the Baltic. The high standard of its monitoring work, and of its anti-pollution proposals (which, once it is signed, will be binding on the participating countries), may well, however, become a blueprint for other future international conventions on marine environments that would cover the whole range of sea-going nations. **Vera Rich**

Women in Helsinki washing carpets in the waters of the port. The seven Baltic states are working to ensure the sea stays clean enough for this spring-cleaning tradition to persist

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NEWS IN BRIEF

US urged to spend more on space manufacturing research

UNLESS the US substantially increases the resources that it devotes to research on manufacturing techniques in space, it could see its national pre-eminence in space activities lost to the Soviet Union and Western European countries, according to a report published by the General Accounting Office, the investigative arm of the US Congress.

The report suggests that the federal government develop a plan for broad collaboration with private industry for research into ways of producing substances such as alloys, crystals, semiconductors, and high purity medicines and vaccines in a space environment. Private industry on its own "cannot be expected to risk high, long-term investments at the present stage of research," the GAO says.

The federal government should be speding two to three times the $20 million currently included in the budget for the National Aeronautics and Space Administration to support such research if it is to retain parity with other industrialised nations, the report says.

Three Mile Island birth defects

OFFICIALS of the Pennsylvania Department of Health confirmed last week that in the last nine months of 1979 13 hypothyroid babies were born in three neighbouring counties compared to an expected number of three such births. The odds against such an event occurring by chance are less than one in 10^5. Thyroid problems including hypothyroidism are known to be associated with exposure to I^{131} an isotope released into the atmosphere during the Three Mile Island accident. Officials said that likelier explanations than radiation exposure could account for the increase but that the timing was "peculiar and curious".

Supreme Court blow to campus unions

THE US Supreme Court dealt a damaging blow to attempts to unionise university faculty members when it ruled last week that an academic's involvement in managerial and administrative activities made him or her ineligible for collective-bargaining protection under federal labour law.

The Supreme Court's judgement was made on a case brought on behalf of the faculty of the Yeshiva University in New York City, by the National Labour Protection Board, which had argued that nothing in their responsibilities as educators precluded faculty members from attempting to resolve concerns about the terms and conditions of employment through collective bargaining.

In a five to four decision, the Supreme Court ruled against the NLPB that because universities had to rely on faculty members to participate in making and implementing decisions, for example over course content or the appointment of new faculty members, they were not entitled to the protection offered to industrial workers.

At present, 80 private universities have agreed contracts negotiated through collective bargaining. The court's decision means that in future university officials may be able to refuse to bargain with faculty unions.

French government starts troubled reactors

THE French Minister of Industry, Andre Giraud, authorised last week the start-up of the troubled French reactors, Gravelines I and Tricastin I. Instruments to monitor the growth of cracks 6mm deep and 7-8mm long in the tubular base plates of the primary circuit heat exchanger have been put in place and the ministry considers that the safety of the reactors is assured.

Dutch aerosol propellants ban imminent

DUTCH authorities expect to ban the use of chlorofluorcarbon (CFC) for aerosol propellants from the end of 1981, the Minister of Public Health and Environment, Dr Ginjaar, has said in reaction to reports from the American National Academy of Sciences (*Nature* 3 January, page 2). The member countries of the European Economic Community (EEC) agreed last year to a 30% reduction in propellant use by 1982. Next year the countries will review the position, and if a total ban is not agreed, the Netherlands will do it alone, possibly followed by West Germany.

According to Dr Ginjaar, only these two countries favour more stringent controls. In the Netherlands the use of CFCs as aerosol propellants have decreased by 30% already, and the authorities are now preparing a complete ban on non-essential uses. **Casper Schuuring**

Information withheld on nuclear waste shipments

KEEPING to the government's nuclear information policy of "no news is good news" (*Nature*, 13 December 1979 page 665), the UK Minister of Transport has refused to produce the names of towns and cities through which nuclear waste is regularly transported by rail. Mr Jack Ashley, MP (Labour, Stoke-on-Trent) received a reply to a parliamentary question from Mr Kenneth Clark, Parliamentary Secretary to the Minister of Transport, stating that the names were being withheld because "it would not be appropriate", to give them. Ashley was told, however, that regular nuclear waste shipments do pass through Stoke-on-Trent.

Nuclear waste transport has been a target for public protest in recent weeks (17 January page 238) causing some official discomfort. At a recent press conference held jointly by the Central Electricity Generating Board and British Rail to explain their position on the shipment of nuclear waste through London, it was revealed that an anti-nuclear campaign plan to stop one of the trains by civil disobedience may have been defeated by information provided by an informer. When asked to elaborate at a later time on the possibility that the CEGB had "its own moles" in the anti-nuclear movement, a CEGB official told *Nature* that the remark was "a light hearted aside" but added that citizens should tell police about wrong doings since "in this country we trust the authorities".

South Africa maintains nuclear weapons capability

A REPORT issued last week* claims that South Africa has developed and maintains a nuclear weapons capability "in order to preserve apartheid in the face of the changing balance of power in southern Africa". Based on a detailed study of South Africa's uranium producing facilities, the report claims that a pilot plant located at Valindaba has the facilities to produce uranium oxide, uranium hexafluoride and enriched uranium. Estimates of plant output since 1975 show that South Africa has accumulated enough enriched uranium to make 4 Hiroshima sized bombs or 12 smaller devices in the 4 kiloton range. Delivery systems would be South Africa's British made Bucanneer and Canberra jets.

In the meantime, confusion exists about responsibility for waht is thought to have been a nuclear explosion off the Cape of Good Hope on 22 September 1979. According to information released by the CIA the explosion has been identified from three independent instruments — the radio telescope in Arecibo, Puerto Rico and two independent observations from the Vela spy satellite. The CIA attributes the bomb to South Africa but a book authored by two Israelis published last week claims that the bomb was an Israeli device exploded with South African facilities.

**South Africa's Nuclear Capability by Dan Smith. World Campaign against Military and Nuclear Collaboration with South Africa. 89 Charlotte Street London W1P 2DQ*

FEATURES

INFCE brings international agreement on nuclear fuel cycle no nearer

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THE International Nuclear Fuel Cycle Evaluation (INFCE) was set up to release growing tension between states over control of the nuclear fuel cycle and the possible spread of nuclear weapons, but, argues **Ian Smart**, without political agreement between governments INFCE alone could not do this

AFTER two and a half years of discussion INFCE has reached its public end with a final plenary session in Vienna. It was bred to be a dispassionate technical analysis of future fuel cycle options in the light of proliferation concerns. How well it has performed will now be tested. Assessments will inevitably vary, if only because different governments looked to INFCE to achieve different things. Especially at the outset, the United States, for example, looked for persuasive evidence that there would be sufficient newly-mined uranium to make reprocessing and the deployment of fast breeder reactors unnecessary for the foreseeable future, and that civil nuclear technology could be developed in 'proliferation-resistant' directions which avoided the presence of separated plutonium.

Others looked for some sort of contrary technical demonstration: that spent fuel would have to be reprocessed to reduce pressure on uranium supply or to make nuclear waste manageable; that plutonium could and should be used in existing thermal reactors; that fast breeder reactors would be needed as quickly as they could be developed; that there were technical means of making any of those activities resistant to proliferation. As the findings of INFCE are studied, it will be found that none of the participants got all it wanted. There is no firm agreement on the future availability of uranium, for example, nor therefore on the rate at which reprocessing should be undertaken or breeder reactors deployed. Indeed, about the only clear conclusions of the technical evaluation are that no conceivable fuel cycle could much reduce the possibility of proliferation and that no single pattern of technical evolution could be appropriate to all national circumstances.

But many of the 46 participating governments have learned something from it on the technical level, and now understand more about the difficulties of both nuclear fuel cycles and proliferation. One part of INFCE's paradox, however, is that technical experts, dealing with issues many of which were essentially economic, contrived, above all, to achieve a political effect. What they demonstrated, in fact, was that the principal issues raised by a possible relationship between civil nuclear fuel cycles and the proliferation of nuclear weapons cannot be resolved by technical ingenuity or arbitrary government intervention, but only by political negotiation and political agreement.

INFCE can best be regarded as a course of drug therapy, administered to the fevered body of international nuclear energy relations. Partly by that means, the patient's temperature has been reduced. The pathology, however, remains unchanged. The prognosis must therefore be that, the drug having been withdrawn, the temperature will rise again unless the basic disease is identified and treated — and the one thing INFCE has achieved in that connection is to establish that both diagnosis and cure must be political.

The one achievement of INFCE which may, perhaps, be forgotten in a flood of technical commentary is that, simply by meeting for more than two years, it has helped to dissipate the fierce antagonisms that have developed among nations in the nuclear fuel cycle trade.

INFCE arose from international turmoil. At the end of the 1960s, the Non-Proliferatin Treaty (NPT) expressed a new international consensus on the need to expand peaceful uses of nuclear energy without increasing the number of countries controlling nuclear weapons. Provided countries without weapons — non-nuclear-weapon states — substantiated their renunciation of them by permitting international inspection, the treaty acknowledged their right to develop nuclear energy for peaceful purposes without further restriction. The NPT was never universally accepted, but, backed by the safeguards system of the International Atomic Energy Agency (IAEA) and supported by a large majority of governments, it offered some hope of setting the general standard for international behaviour. Five years later, however, both the standard and the hope lay wounded.

> 'The temperature has been reduced . . . but the pathology remains unchanged'

In 1974, India, which had not accepted the NPT, tested its first nuclear explosive. Almost simultaneously the Nixon Administration offered power reactors to Egypt and Israel, despite the fact that neither was a party to the NPT. Another nuclear-weapon state, France, having refused to sign the NPT, was negotiating sales of reprocessing technology to both Pakistan (which had also rejected the NPT) and South Korea. West Germany, agreed to provide yet another NPT non-party, Brazil, not only with reprocessing technology but also with equally 'sensitive' equipment for uranium enrichment. And meanwhile the oil crisis was exciting interest in nuclear power and persuading many of those committed to it to seek self-sufficiency.

Shocked by the Indian experience, and fearful that their commercial rivalry, if uncontrolled, might encourage a dangerous increase in the number of countries able to produce weapons, the major governments involved in nuclear exports formed the Nuclear Suppliers Group (NSG) and (secretly) agreed in 1975 on a set of guidelines to govern the export of 'sensitive' items. Although the suppliers eventually published these rules, in 1978, the fact that they had drawn them up in secret and without wider consultation aroused the resentment and apprehension of their customers, especially in the Third World. As to the substance of the NSG guidelines it was soon recognised that they went beyond the NPT itself, by calling for restrictions on the international transfers of certain allegedly 'sensitive' fuel cycle technologies such as enrichment and reprocessing, by limiting the use which those who did import such technology might make of it, and by recommending

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Ian Smart is an adviser on international energy affairs

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that exporters of nuclear fuel should retain the right to veto any reprocessing of it after use.

There was a widespread impression that the guidelines discriminated against the less developed nuclear countries, and that, by implying such countries had no general right to enrich uranium or reprocess spent fuel, even under safeguards, they made nonsense of a fundamental NPT concept. The NPT consensus had been founded on the idea that, for all non-nuclear-weapon parties, all peaceful and safeguarded nuclear activities were equally legitimate. Now it was said that some non-nuclear-weapon states — and some peaceful technologies — were more equal than others.

These resentments were compounded in April 1977, with President Carter's announcement that the US would indefinitely defer reprocessing and the use of plutonium, and would also hold back development of fast reactors capable of 'breeding' plutonium. At the same time, it would withhold approval for foreign reprocessing of US-origin nuclear fuel. Such a policy, subsequently reinforced by the US Congress in the Nuclear Non-Proliferation Act, could be seen as a logical extension of the NSG guidelines. It now struck, however, not only at the transfer of reprocessing (or enrichment) technology but also at the conduct of reprocessing by those who already possessed the capacity: by Britain and France, which had plants, and by West Germany and Japan, which were seeking to build them. The effect was to divide the suppliers against themselves.

The overall result, by the spring of 1977, was that commercial and political confidence in the whole field of international nuclear relations was at a nadir. Contracts had been suspended. Bilateral agreements had been unilaterally recalled, in mid-term, for renegotiation. Multilateral agreements, such as the NPT, had become playthings for minority interpretation. North-South nuclear relations were already embittered, and relations between developed countries were now becoming openly acrimonious. Some cooler review of proliferation issues was urgently needed, if rampant frictions were not to destroy the fabric of international cooperation and trade.

So INFCE was conceived at the the May 1977 Economic Summit in London, and officially born in October of that year in Washington. Its eight working groups, with the Technical Co-ordinating Committee (TCC) composed of their 22 co-chairmen, have since held 70 meetings and produced 20,000 pages of commentaries and drafts. Now it is winding up, with a final plenary session whose task this week is to set its seal on the lengthy 'Overview' agreed in the TCC and on the even longer reports of the separate working groups. And next come the consequences of its three years' work.

> 'Nothing less than the world pattern of nuclear energy development is at stake'

> 'As the findings of INFCE are studied, it will be found that none of the participants got all they wanted'

In the short term, international nuclear relations will inevitably come under renewed pressure. In several countries — Sweden and West Germany, for instance — the link between nuclear power and proliferation is an immediate issue in domestic politics. In several cases, as between the United States and Japan or the United States and Euratom, bilateral arrangements for nuclear cooperation and trade must now be re-cast in more restrictive form. And in August 1980, at the multilateral level, parties to the NPT must meet at their second Review Conference, where the political tensions unresolved by INFCE will again be prominent. Unless those and other immediate stresses are successfully absorbed, the temperature of international nuclear relations could easily be rising again by the end of the year.

Looking to the longer term, the problem is simpler but more difficult. What has collapsed since 1974 is not some set of formal rules or standards but rather the sense of international confidence: confidence that nuclear power can be used more widely without intolerably increasing the risk or fear of weapons proliferation; confidence that international contracts and agreements will be fulfilled, and that all those who seek the energy benefits of nuclear power will be able to draw equitably on international trade under safeguards.

As a result, nothing less than the world pattern of nuclear energy development is at stake. On one side, there is a model of development in which countries committed to nuclear energy programmes, but unconvinced that essential goods and services will always be available on acceptable terms, are driven to seek nuclear autarky as the only other route to energy security. With little regard to cost, more of them will then for instance, acquire their own plants for enrichment and reprocessing.

On the other side is a model of inter-dependent development, in which the same countries, offered a reliable system of nuclear trade and cooperation, will voluntarily optimise their programmes within a regional or global context. Political and economic rationality will then coincide to produce an efficient international distribution of labour in the nuclear fuel cycle, including its 'sensitive' stages.

The former pattern of 'fragmented autarky' would clearly generate greater fears of proliferation, while also being resistant to internationally agreed regulation or safeguarding. The paradox of some supplier policies since 1974, however, is that, by seeking to restrict nuclear trade and confine fuel cycle technology and capacity to a few countries in the name of non-proliferation, they have reduced the confidence of other states in inter-dependent development and actually increased the pressure on them to move to 'fragmented autarky', making the system progressively less amenable to international regulation.

The privately sponsored International Consultative Group on Nuclear Energy, in its recent report, has called for "a new 'bargain of confidence', in which credible guarantees of peaceful purpose will be balanced by firm assurances of access to nuclear services, materials and technology". Such a bargain of confidence can only be achieved by political negotiation. Non-proliferation treaties and international safeguards provide a starting point, and INFCE may offer some signposts. Experience of the last decade proves, however, that more is needed: some general code of conduct for international nuclear trade, agreed by importers as well as exporters, and possibly some more imaginative institutional arrangements for the international management of 'sensitive' activities and materials.

The alternative is not to be contemplated. Ten years ago, governments made the mistake of turning away from the NPT before its general provisions had been converted into concrete and equitable arrangements suited to specific international circumstances. INFCE has solved nothing, but it has bought an opportunity to repair some part of that gross error. If governments, especially in the advanced nuclear countries, fail to build onwards from INFCE into a lattice of political negotiation, design and agreement, they will have only themselves to blame if, in another few years, international nuclear relations are again as inflamed and painful as they were when it started. □

In *Nature* next week Sir Hermann Bondi, chief scientist at the UK Department of Energy and leader of the UK delegation to INFCE over the past two years, explains how INFCE reached its technical conclusions.

CORRESPONDENCE

Iraq's efforts to reverse the brain drain

SIR, — I was very pleased with Ziauddin Sardar's article in *Nature* (January 24, page 327) in which the author goes into depth in an effort to describe the exporter and importer countries of scientists and the problems these scientists face in the host countries. Although the report reflects the realities in these countries, it failed to mention anything about Iraq, a country that has a very clear policy in attracting expatriate scientists to return and establish residence in Iraq. In fact as early as 1974, the Revolutionary Command Council of Iraq instituted the "law of return" to any Arab scientist, engineer or holder of a high university degree to return to Iraq. The government, on its part, provided the returning scientists with several enticements including:

- all the facilities needed to do science
- an option to acquire the Iraqi citizenship after a year's stay
- a lot of land to build a house
- a substantial, long term, interest-free loan to effect the building of the dwelling.

Iraq's ambitions to foster science and technology is evident by the construction of new universities and science centres, in recent years, to accommodate the returning scientists as well as to accommodate the ever-increasing number of graduates sent to various developing countries for higher studies and training.

The development of science and technology receives support at the highest levels. Indeed Mr Saddam Hussain, the President of Iraq, gave his clear instructions to do what it takes to develop the scientific base in Iraq. He personally authorised the establishment of a new programme of short-term training seminars to be held in Iraq at government expense. The trainees are Iraqi and other Arab nationals and the training staff are scientists residing in the western world. I personally, along with my colleagues of the Arab American University Graduates (AAUG) had the opportunity to conduct some of these seminars in Baghdad, under the auspices of the Ministry of Health.

Because of its pan Arab nationalistic policy, the government of Iraq insists that "Iraq is for Arabs". This policy is translated into action by waiving the visa requirements to any Arab national, wherever he lives, and who wishes to visit, work or establish a home in Iraq. To my knowledge this is the only Arab country that has felt secure and abolished the visa requirements. To further promote the feeling that Iraq is for Arabs, the non-Iraqi Arab nationals are treated as equal to their Iraqi counterparts. The treatment of "second class citizens" of the expatriates mentioned in Sardar's article is non-existent in Iraq. One can safely deduct that Iraq's long-term ambitions are to bring Baghdad to its golden days as a centre of science and technology.

I am neither an Iraqi national nor a member of the ruling political party, but a concerned Arab scientist residing in the United States and currently on short leave in Switzerland.

ABDALLAH M. ISA
Institut für Klinische Immunologie, Bern, Switzerland

Defending democracy or something else?

SIR, — Your correspondent (Rolf Seifert, 31 January, page 426) alludes to the "suicide of democracy" if "disruptive Marxists" are allowed to occupy "influential positions" in society. According to cases we are actually involved with, "influential positions" include postmen, railwaymen, teachers of educationally subnormal children and civilian army cooks as well as the cases quoted in *Nature* (6 December, page 549).

It is the suicide of democracy for the state to monitor legal involvement in the democratic process, to label them arbitrarily as 'disruptive', and then continue to use them as a criteria for the dismissal of long-standing public employees from their jobs. When you defend democracy by such methods, you do not defend democracy, you defend something else.

There is an old English proverb about killing the goose that lays the golden eggs.

C. N. M. POUNDER
National Campaign against the Berufsverbote, Edinburgh, UK

Nuclear safety conference open to the press

SIR, — In "News in Brief" (24 January, page 326) you mention the IAEA's International Conference on Current Nuclear Power Plant Safety Issues, to be held in Stockholm from 20-24 October 1980, with a misleading title. I would like to make the following points:

- This conference, like all others organised by the Agency, is open to the press and to the public, accommodation in the conference hall permitting.
- The requirement that papers be submitted by governmental designees is a standard requirement for all meetings of the United Nations and its agencies, since they are intergovernmental bodies, and in practice, therefore, these papers represent a wide range of opinions.

GEORGES DELCOIGNE
International Atomic Energy Agency, Vienna, Austria

Makerere University library appeal

SIR, — We would like to bring attention to some of the difficulties currently being faced by Makerere University in Uganda, particularly with regard to the university's library. All finance for the library was cut off in 1974, and in the last few months since the liberation only a portion of it has been restored. In consequence all runs of the 2000 or so learned journals which the library used to take ceased, as did the purchase of new books. Moreover during the recent fighting many books and much library equipment were looted.

A university cannot function effectively without a good up-to-date library, and teaching and research must both be severely hampered. Makerere used to have one of the best libraries in East Africa, housed in a beautiful building, and both of us have in the past spent many peaceful hours working in it. We have therefore decided to open a fund to help re-equip the library, and we appeal to all those with an interest in Makerere and a concern for education in Africa to contribute generously. We will ensure that the fund is used for the designated purpose by consulting with the librarian and then purchasing and despatching books to him. If you would like to help please send a cheque made payable to the "Makerere Library Fund" to one of us at the address below.

PATRICK MANGHENI
PETER MILLER
The Queens College, Oxford, UK

Maoist dogma clashes with fair appraisal of science

SIR, — I deplore the descent of *Nature* into the murky realms of propaganda. A series of feature articles on Chinese science by Tong B. Tang published in the 31 January, 7 February and 14 February issues use the most blatant political jargon, something totally unsuited to a scientific periodical. The epithet 'Gang of Four', a political insult which would not be out of place of George Orwell's book *1984*, 'revisionism', 'capitalist roaders' and other jargon, combined with Maoist unintelligibles like "one divides into two" combine to make the articles unpalatable. Surely the peculiar outpourings of Maoist dogma clash with a fair and dispassionate appraisal of progress in Chinese science. The 'ascent of deviationists' may be of interest to the changing policies of Marxist doctrinal quibblers, but such extravagant phraseology masks what is really the issue — the use of science for political ends. Like so-called Islamic science or even Christian science, these 'sciences' which are oriented to some prearranged goal like the 'dialectics of nature' serve only to bolster the preconceptions and prejudices of dogmatic plutocrats.

NIGEL PENNICK
Cambridge, UK

Critic of "The Sirius Mystery"

SIR, — Robert K. G. Temple's long letter (17 January, page 242) seems to have been, in part, motivated by a sense of indignation at not having been appropriately cited by Rowan-Robinson (in his review of Carl Sagan's "Broca's Brain", (8 November, page 176) nor by Carl Sagan (in his book "Broca's Brain").

It is therefore worth pointing out that Temple himself carefully avoids any reference to articles critical of the claims he makes in his book "The Sirius Mystery." One such article that readers of *Nature* might wish to know about is Pesch, P. and Pesch, R. *The Observatory,* **97,** 26; 1977.

PETER PESCH
Warner and Swasey Observatory, East Cleveland, Ohio, US

Radical test

SIR, — With reference to the Shirley Williams interview (3 January, page 8), it is odd she should choose herself as "litmus paper" while her colleague, Mr Prentice, so readily changed from red to blue. It seems he failed the acid test of being a radical.

D. P. HAWORTH
Department of Botany, University of Illinois, US

Transposable mating-type genes in yeasts

from Urs Leupold

THE genetic analysis of the inheritance of yeast mating types has developed recently from a rather specialised subject studied by only a small group of yeast geneticists into one of central genetical interest. This is due to the growing realisation that the mating type switches observed in both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are yet another example of the phenomenon of transposable or translocatable genes whose importance is increasingly being recognised throughout the living kingdom.

Saccharomyces cerevisiae

In *Saccaromyces cerevisiae* there are two mating types *a* and *α*. Vegetative haploid cells of opposite mating type can fuse to form vegetative diploids which may subsequently sporulate, producing haploid spores. In homothallic strains of *S. cerevisiae* haploid cells of one mating type can 'switch' mating type during vegetative growth, giving rise to progeny of the opposite mating type.

In *S. cerevisiae,* the results of the genetical analysis of mating-type interconversions in homothallic and heterothallic strains have led I. Herskowitz and coworkers to develop a hypothesis known as the 'cassette model' to explain the observed interconversions (reviewed by I. Herskowitz *et al.* in *The Molecular Genetics of Development,* ed. Loomis, W. & Leighton, T., Academic Press, 1979). Briefly, this model proposes that the switching between the two opposite mating types *a* and *α*; which occurs as frequently as every cell division in homothallic strains, is due to the transposition of *α* and *a* alleles from silent storage sites to the mating-type locus where they replace the allele present, either *a* or *α*. The storage sites carrying either an *a* or an *α* allele in an unexpressed form have been characterised genetically as two gene loci *HML* (carrying the *α* or, less frequently, the *a* allele) and *HMR* (carrying the *a* or, less frequently, the *α* allele). The two loci map far from the mating-type locus *MAT* but in the same chromosome on opposite sides of *MAT* (*HML* in the left and *HMR* in the right arm) (see Fig. 1). In the presence of the *HO* gene for homothallism which allows for frequent switching (as opposed to the rare mating-type interconversions observed in heterothallic strains of constitution *ho MATa* and *ho MATα*), defective mutant alleles of the type *mata*$^-$ or *matα*$^-$ are quickly replaced by the corresponding functional alleles which are stored in a silent form at the *HML* and *HMR* loci. Mutations induced in the *HML* and *HMR* loci can also be transposed to the *MAT* locus by switching. This transposition is a long-range transfer and seems to occur also in the *trans* configuration between different (homologous) chromosomes.

A recent paper by J. Hicks, J.N. Strathern and A.J.S. Klar (*Nature* **282**, 478; 1979) offers physical evidence for the existence of homologous sequences at the *MAT, HML* and *HMR* loci which is entirely consistent with the cassette model. Moreover, it shows that this homology is not restricted to alleles which determine the same mating type (for example *MATα, HMLα, HMRα*) but that even alleles determining opposite mating types (for example *MATα, MATa*) share homologous sequences. DNA restriction fragments carrying an *α* allele of either *MAT, HML* or *HMR* differ however from restriction fragments carrying *a* alleles in being uniformly about 200 base pairs larger. Both homothallic (that is, frequent) and heterothallic (rare) mating-type interconversions involve a corresponding change in size of the restriction fragment carrying *MAT*. This shows that the

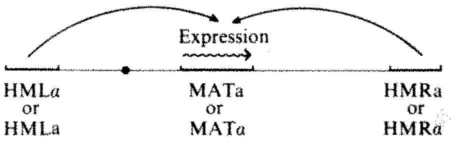

Fig.1. Relative arrangements of HML, MAT and HMR on chromosome III of *S. cerevisiae.* During mating type interconversion a copy of *a* or *α* is inserted into the MAT locus where it is expressed. The sequence previously located at the MAT locus does not reappear in the genome and is presumably lost. (After Hicks *et al. Nature op. cit.*)

interconversion process involves a rearrangement of the DNA sequences present at the *MAT* locus, most probably the substitution of an *a* or *α* sequence by a partially homologous *α* or *a* sequence from one of the silent genes *HML* or *HMR* as predicted by the cassette model.

These remarkable results have been obtained by cloning a 6.6 kb *Bam*H1 restriction fragment carrying an *α* allele sequence (the *HMLα* allele) on a plasmid capable of replicating in both yeast and *E. coli* and of transforming yeast at high frequency. The cloned sequence was identified by its ability to complement a defective *mat* allele for both mating and sporulation when plasmid DNA carrying the *α* sequence was introduced into cells carrying the mutant allele at the *MAT* locus. When this plasmid was used as a hybridisation probe against Southern blots of restricted DNA from a series of different yeast strains carrying known mating-type alleles at the mating-type locus *MAT* and the two silent loci *HML* and *HMT,* characteristic differences in restriction patterns between *a* and *α* strains were observed. In tetrad analyses of meiotic crosses between strains differing in their mating-type constitution at either the

Urs Leupold is Professor of Microbiology at the University of Berne.

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mating-type locus or one of the silent loci, these differences segregated along with the mating-type alleles studied.

Klar, Hicks, Strathern and J. Broach have presented these results at a meeting on the Molecular Biology of Yeast which took place in Cold Spring Harbor last year. There they presented additional evidence (not mentioned in the paper) based on heteroduplex mapping which shows that the *α* allele differs from the *a* allele not only in the addition of a sequence of about 200 base pairs but also in the substitution of an adjoining sequence of about 700 base pairs. The heterologous region (700 bp in *a* and 900 bp in *α*) is flanked on both sides by homologous regions which permit hybridisation between complementary *a* and *α* strands.

These findings have independently been confirmed and extended by K.A. Nasmyth and K. Tatchell who have cloned the *α* sequence from the *MAT* locus and who have also discussed the results of a heteroduplex analysis of the three mating-type genes at the same meeting. A paper presenting their data will shortly be published (*Cell,* in press). A heteroduplex analysis of the six possible combinations between purified *Hin*d III fragments carrying *MATα, MAT*a, *HMLα* or *HMRa* showed that the homologous regions common to all loci are confined to two blocks of DNA, one of 230 base pairs located on one side (the 'left' side) and one of 700 base pairs located on the opposite side (the 'right' side) of the *a/α* specific sequence of approximately 800 base pairs (700 bp in *a* and 850 bp in *α* according to their data). The *a* and *α* specific insertions are present in the same orientation, with respect to the two homology blocks, in active and silent loci. *HMRa* lacks an extensive 700 bp DNA sequence to the right of the large right hand homology block and probably also a small (100 bp) sequence to the left of the small left hand homology block, both of which are present at *MAT* and *HMLα* (see Fig. 2). In *MATa* and *MATα*, the regions of homology flanking the *a/α* specific sequence extend to the ends of the 4.1 kb and 4.3 kb *Hind* III fragments carrying the *a* and *α* alleles of the *MAT* locus.

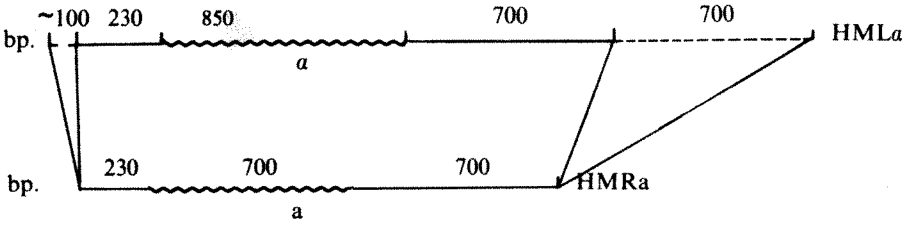

Fig.2. Schematic representation of the differences between HMLα and HMRa (see text for explanation).

On the basis of these data, the extent of the sequence transposed from *HMRa to MATα* in an *α→a* switch seems to be at least 700 bp and at most 230 + 700 + 700 = 1,630 bp. In an *a→α* switch, the sequence transposed from *HMLα* to *MATa* consists of a minimum of 850 bp but may include as many as 100 + 230 + 850 + 700 + 700 = 2,580 bp. The extensive sequence homology shared by the active mating-type locus *MAT* and the silent loci *HML* and *HMR* suggests that the interconversion replacing the resident mating-type sequence at the *MAT* locus by an alternative sequence from one of the two silent genes may occur by a mechanism of non-reciprocal gene conversion. This would involve an intrachromosomal alignment of active and silent loci of opposite type, followed by the transfer of a single-strand DNA segment from the silent to the active locus where it replaces the corresponding segment including the central sequence of opposite mating-type specificity. The resulting heteroduplex structure containing an unpaired loop in the centre of the MAT locus could then be resolved either by local excision repair which replaces a DNA segment of the resident *MATa* or *MATα* strand by a complementary copy of the transferred *HMLα* or *HMRa* single-strand segment, or by normal DNA replication with leads to the segregation of two daughter chromosomes carrying alleles of opposite mating-type specificity at the active mating-type locus. The recent finding of R.E. Malone and R.E. Esposito (also reported at the Cold Spring Harbor yeast meeting mentioned above and see *Proc. natn. Acad. Sci. U.S.A.* **77**, 503; 1980) that a DNA repair mutant defective in both meiotic and mitotic recombination (*rad*52-1) prevents homothallic switching, lends direct support for the involvement of generalised recombination functions in the mating-type interconversions observed in yeast.

Together, the two reports from the groups of J. Hicks and coworkers in Cold Spring Harbor and of K.A. Nasmyth and K. Tatchell in Seattle represent the first physical evidence confirming the genetical evidence that the two silent mating-type loci *HML* and *HMR* show an extended sequence homology with the active mating-type locus *MAT* and with one another when carrying alleles of the same mating-type specificity, either *a* or *α*. They demonstrate further that even when carrying alleles of opposite mating-type specificity, the three loci share homologous sequences although their *a* and *α* alleles differ in a central region of 700 and 850 base pairs, respectively. Together with the genetical evidence obtained by the group of I. Herskowitz at the University of Oregon and by many others, they provide very strong support for the cassette model which proposes that the mating-type interconversions in yeast are due to the transposition of mating-type alleles from silent storage sites ('cassettes') to the mating-type locus where they become expressed.

The mating-type locus plays a key part in the differentiation of yeast cell types for it controls not only mating but also sporulation. Diploid cells homozygous for the *a* or *α* allele at the mating-type locus *MAT* can mate with cells of the opposite mating type but are unable to undergo meiosis and sporulation. Diploid cells of heterozygous constitution *MAT*a/*MATα* can be induced to undergo meiosis and sporulation but are unable to mate with either *a* or *α* cells. An understanding of the role of gene transposition in the differentiation of these yeast cell types may well contribute to a future understanding of cell differentiation in higher eukaryotes (see I. Herskowitz *et al. op. cit.* 1979).

Schizosaccharomyces pombe

Mating-type switching in *Schizosaccharomyces pombe* (see Egel *Nature* **266**, 172; 1977), although still in the purely genetic phase of research, has recently been shown to resemble 'cassette-recording' in *Saccharomyces,* partially at least (R. Egel, Copenhagen, as presented at the Cold Spring Harbor meeting). One of the two closely linked mating-type genes found in this species, *mat2-P,* has turned out to represent the silent store of 'plus' information, which can only be activated by copy-transposition to *mat1* — the 'mating-type locus'. The 'minus' information residing at *mat1-M* beforehand is formally replaced by the inserted *P* copy, but whether this is merely due to transient separation of *M* from its controlling signal or whether the *M* segment is, in turn, excised is still unknown. In the latter case another gene of silent *M* storage should exist elsewhere, for which there is as yet no evidence. A major difference remaining (relative to *Saccharomyces*) lies in the fact that this *P* transposition requires the close linkage here encountered and does not function in the *trans* configuration. This linkage and the *cis* effect have hitherto obscured the activation mechanism of the *P* gene, which only revealed itself after mutants had become available that reduce the naturally high frequency of switching. In such mutants the activated (transposed) *P* copy remained in place long enough to be detected. Otherwise it was lost too soon (within 1-3 cell divisions) by the next switch-back reaction. □

Interstellar grains in meteorites?

from R. Hutchison

TODAY, the most favoured theory of the origin of the Solar System entails the collapse of a gas-dust cloud, perhaps initiated by a shock-wave from a nearby supernova. Heating during the collapse phase would have vaporised much, if not all, of the pre-existing dust. The question then arises: did any of the presolar dust-grains survive? And if so, how might they be recognised? Answers to these questions may be found in carbonaceous chondrite meteorites.

Evidence that some materials are enriched in the products of a supernova (or supernovae) was found in inclusions in the Allende meteorite. The scientific community was extremely lucky to receive two tonnes of specimens from the Allende meteorite shower that fell in northern Mexico in February, 1969, making available for study kilograms of samples of a type which had previously been doled out in gram-sized amounts. The Allende material was found to contain whitish inclusions consisting of minerals rich in calcium, aluminium and titanium. Such mineral assemblages were soon recognised as those predicted by Lord in 1965 (*Icarus* **4**, 279) as the first material to condense from a cooling gas of solar composition. And in 1973, R.N. Clayton *et al.* (*Science* **182**, 485) established that the oxygen of the high temperature inclusions and chondrules of Allende and related carbonaceous chondrites is enriched in pure ^{16}O. It was argued that the pure ^{16}O was produced by nuclear processes in an expanding outer shell of a supernova. Soon after the pure ^{16}O was introduced into the Solar System, condensation of refractory minerals trapped a portion of the pure isotope, the remainder having been mixed in with average Solar System oxygen, so losing its identity.

Further studies revealed that the white, calcium, aluminium, titanium-rich inclusions of Allende have anomalous isotopic abundances of various elements. Potentially, the most important of these anomalies is in ^{26}Mg because this is the daughter isotope of ^{26}Al, of which the half-life is only 740,000 years. The ^{26}Mg anomaly was discovered by Gray and Compston (*Nature* **251**, 495; 1974) and by Lee and Papanastassiou (*Geophys. Res. Lett.* **1**, 227; 1974). Wasserburg's group soon showed that in several Allende inclusions there is an internal isochron relationship between ^{26}Mg/^{24}Mg and ^{27}Al/^{24}Mg (*Geophys. Res. Lett.* **3**, 109; 1976). The isotope ^{27}Al is the only stable isotope of the element, and ^{24}Mg is the most abundant common isotope of magnesium. Thus, in each mineral of some inclusions there is a positive correlation between ^{26}Mg and the aluminium to magnesium ratio. We can therefore conclude that these inclusions crystallised when ^{26}Al was still extant. But, as D.D. Clayton has pointed out, there is no positive proof that the white inclusions crystallised within the Solar System (see for example *Nature* **257**, 36; 1975). Because isotopic anomalies of different elements, but especially light and heavy elements, do not generally correlate with each other, it is argued that crystallisation of the white inclusions must predate the Solar System. Injection of newly synthesised nuclides from a supernova into a presolar gas-dust cloud should have caused complete mixing of the injected elements. Thus, any isotopic anomalies should be correlated within each white inclusion. Lack of such correlation may be interpreted as indicating that the white inclusions represent condensates from the outer shells of the expanding supernova itself, assuming that the shells are blown concentrically outwards with a minimum of mixing between them.

Last year, a presolar age for two Allende inclusions was given a boost. Jessberger and Dominik (*Nature* **277**, 554; 1979) found that two inclusions have ^{39}Ar–^{40}Ar ages of about 5.0 Ga. The technique uses the ^{40}K–^{40}Ar decay scheme, but ^{40}K is estimated from the amount of ^{39}Ar produced by neutron irradiation of ^{39}K. If the ^{40}K/^{39}K ratio throughout the inclusion had been uniformly high, it could have accounted for the apparently old age. However, Stegmann and Begemann (*Nature* **282**, 290; 1979) found the potassium isotopic ratios to be normal. Thus the inclusions seem to have crystallised 400 Ma before the birth of the Solar System. But because other age determination techniques give a 'normal' age of about 4.55 Ga for Allende and its inclusions (see for example Tatsumoto *et al. Geochim. cosmochim. Acta* **40**, 617; 1976 and Chen and Tilton, *ibid.*), the ^{39}Ar–^{40}Ar age is still treated with suspicion. Even so, there seems to be a good chance that in some of the Allende inclusions we have presolar aggregates of refractory minerals up to about 1 cm in diameter.

Other approaches in the search for presolar, interstellar grains have been taken by Anders' group at Chicago and Eberhardt's at Bern. In 1969, Black and Pepin argued by a process of elimination than an anomalous neon component in some meteorites is of presolar origin (*Earth planet. Sci. Lett.*, **6**, 395; 1969). This type of neon, 'Ne-E', is characterised by low ^{20}Ne/^{22}Ne and ^{21}Ne/^{22}Ne ratios; Ne-E is, therefore, highly enriched in ^{22}Ne. Because noble gases are released from mineral grains at moderate temperatures, any carrier of Ne-E could not have had a high temperature history like that of the Allende inclusions. So, if the carrier minerals of Ne-E could be separated and identified, this might provide a sample of interstellar grains. To this end the Chicago group used chemical separation techniques and the Bern group physical methods.

Lewis *et al.* of Chicago (*Astrophys. J. Lett.* **234**, 165; 1979) used a sample of the water-bearing, CM2, carbonaceous chondrite which fell at Murchison, Victoria, Australia in September, 1969. As with Allende, scientists were fortunate to have been provided with about 500 kg of a type of meteorite which had previously been in short supply. The Chicago group dissolved away over 99.7% of their sample, using various reagents including HF, HNO_3, NaOH and H_2O_2. The residue was split into three size-fractions and from aliquots of each the rare gases were extracted by stepwise heating. Isotopic ratios were measured in the gas released at each heating step. Ne-E was released from the coarse (>3μm) fraction in two steps at 800 °C and 1,200 °C. Low-temperature (800 °C) release was not observed in an aliquot of material which had been treated with $HClO_4$. The low temperature carrier destroyed by $HClO_4$ was thought to be an oxidisable, carbon-rich material. From previous work and from its resistance to attack, the high temperature carrier of Ne-E was tentatively identified as spinel ($MgAlO_4$). In addition to carriers of Ne-E, a carrier of *s*-process xenon was identified as a carbon-rich phase stable at 1,400 °C. Lewis *et al.* concluded that the two carbonaceous phases and spinel are 'extrasolar'. However, stability of spinel requires a more oxidising environment than phases rich in elemental carbon. The authors, therefore, speculated that the extrasolar minerals may be condensates from red giants "at different stages of evolution".

The Bern group prepared nine density fractions of the Orgueil, water-bearing, carbonaceous chondrite, and stepwise heating was used on all but one, which was too small (*Astrophys. J. Lett.* **834**, 169; 1979). Ne-E was most highly concentrated in the least dense fraction (<2.3 g cm^{-3}), from which it was released below 900 °C. From three high density fractions (total range, 2.9–3.6 g cm^{-3}) Ne-E was mostly released at about 1,200 °C. Because potential contaminants are of lower density, it was argued that the Ne-E carrier probably has a density of about 3.5 and is probably spinel. Eberhardt *et al.* point out that the low density fraction has a low content of cosmic ray-produced ^{21}Ne, which indicates a low abundance of the target elements Mg, Al and Si, and is consistent with a carbon-rich composition. Furthermore, the composition argues against the possibility of the *in situ* production of pure ^{22}Ne by particle bombardment within the Solar System. A presolar origin of one Ne-E carrier therefore seems assured. □

R. Hutchison is in charge of the national collection of meteorites in the British Museum (Natural History), London.

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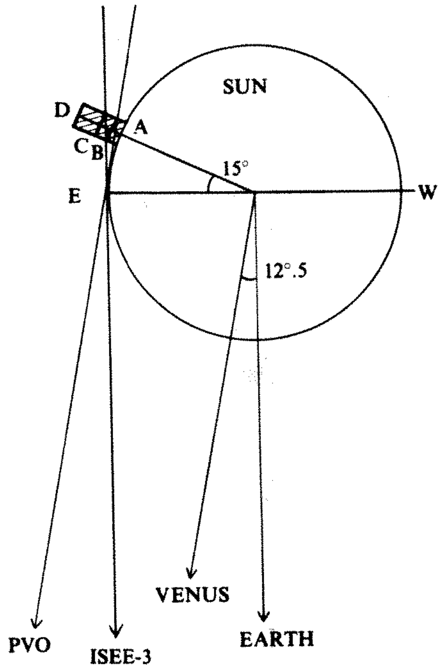
The hard X-ray Sun in stereo

from John C. Brown

VIRTUALLY on the eve of launch (14 February, 1980) of the first ever solar hard X-ray imaging telescope, aboard NASAs Solar Maximum Mission (SMM), a group of US researchers has scooped some of its expected results without the use of imaging instruments. Kane and Anderson of Berkeley and Evans, Klebesadel and Laros of Los Alamos (*Astrophys. J. Lett.* **233,** L151; 1979) have obtained quantitative information on the spatial structure of a solar hard X-ray source by simultaneous observations from two separate spacecraft. Their results provide invaluable insight into the process of electron acceleration which is central to the solar flare problem.

At the time of observation (5 October, 1978 around 0632 UT) an International Sun Earth Explorer (ISEE-3) was located near the Earth while Pioneer Venus Orbiter (PVO) was 12.5 degrees eastward as seen from the Sun (see Figure). Fortuitously, a flare occurred in an active region A some 15 degrees behind the solar limb which consequently occulted different parts of the flaring solar atmosphere from the two spacecraft. As a result (see Figure) ISEE-3 observed only X rays emanating from altitude (C–D) in the source greater than 25,000 km above the photosphere (A) while PVO observed all emissions (B–D) from above 700 km altitude. At burst peak the PVO flux above 50 keV photon energy exceeded the ISEE flux, extrapolated to the same energy range, by a factor of 600 and had a spectrum harder by about +2 in the power-law exponent. We can thus conclude that the hard X-ray source is concentrated at altitudes below 25,000 km, particularly at higher photon energies, with a factor of 600 above 50 keV. This result is a great advance on previous behind-the-limb results from single spacecraft which established the existence of a high altitude source component but gave no information on the differential height structure.

The importance of hard X-ray bursts lies not in the bremsstrahlung X rays themselves but in the energy of the electrons emitting them. These electrons represent a significant, and perhaps a large, fraction of the total energy released by magnetic field dissipation in a solar flare. How severe are the demands that this places on the electron acceleration process depends strongly on the hard X-ray source model assumed. In particular the acceleration efficiency involved is modest if the bulk of the source electrons are in a relaxed quasi-thermal state. On the other hand, if the source electrons are non-thermal they lose most of their energy by collisions to the cooler thermal plasma rather than to bremsstrahlung and a far greater total electron energy is required.



Schematic showing directions of the two spacecraft as seen from the Sun and the different heights in the X-ray source (hatched area) seen from each spacecraft.

Two possible cases of the latter situation are the descending electron beam (thick target) model (with acceleration near D in the Figure) and the trap model where fast electrons are confined in a magnetic arch after acceleration. Although these latter models pose problems for acceleration mechanisms, they provide a plausible interpretation of impulsive chromospheric emissions in flares in terms of electron bombardment. The greatest single source of ambiguity in modelling hard X-ray bursts to date has been the lack of spatial resolution.

There seems to be no ready interpretation of Kane *et al.*'s results in terms of the trap model since this predicts comparable X-ray fluxes from the trapped (coronal) electrons and those precipitating down the limbs to the chromosphere (unless by chance ISEE just glimpsed part of the arch summit at C). A thermal model just might fit the facts if quasi-thermal emission emanated from CD at energies up to around 50 keV while the high energy electron tail escaped downward. The most convincing interpretation at present, however, is the thick target model which predicts a difference of +2 in spectral index between coronal and chromospheric components, and much greater total flux at low altitudes (Brown & McClymont *Solar Phys.* **41,** 135; 1975). The PVO/ISEE flux ratio would then require an acceleration site D located at a plasma column depth of 2.5×10^{18} protons cm^{-2} above the 25,000 km level C. For quiet Sun conditions the plasma density n here is around 10^8 cm^{-2}, but such a low density could not sustain a stable reverse current to neutralise the descending beam. Reverse current stability demands $n \gtrsim 5 \times 10^9$ cm^{-3} which is consistent with the density enhancements typical of active regions. It will be of great interest to see whether a similar source distribution with height occurs in other bursts or if these are more consistent with one of the other source models.

In the coming months SMM will disgorge vastly more hard X-ray source spatial data than are contained in Kane *et al.*'s results. Specifically it will yield detailed information on horizontal source structure and so test whether electrons really do bombard the chromosphere. On the other hand the SMM telescope is limited to photon energies below 30 keV and, furthermore, has no spatial resolution along the line of sight. Thus the 'stereo' data of Kane *et al.* will remain unique until we are again fortunate enough to have two spacecraft and a flare in the right places at the right time. ☐

J.C. Brown is a Senior Lecturer in the Department of Astronomy, University of Glasgow.

Tailoring liposome structure

from Gregory Gregoriadis

THE idea that drugs entrapped in lipid envelopes (liposomes) may provide a better means of delivery to specific sites within the body has aroused wide interest. Much of the interest in the liposomal carriers has no doubt stemmed from the almost unlimited number of potential versions in terms of composition, size and other characteristics. Indeed, correct choice of these parameters has, in some instances, led to liposomal preparations best suited for optimal drug action (for reviews of various aspects of liposomes see *Liposomes and their uses in Biology and Medicine* (D. Papahadjopoulos ed.) *Ann. N.Y. Acad. Sci.* **308,** 1978; *Liposomes in Biological Systems* (eds G. Gregoriadis & A.C. Allison) John Wiley and Sons Ltd, 1980). Recently, attempts have been made to rationalise liposome development by tailoring their structure to the particular biological milieu in which they are intended to act.

The ability to control the extent to which liposomes in the blood retain their entrapped drugs is obviously important to those interested in applying them systemically. Although in many cases drugs must be transported intact to where they are needed, in others their gradual release in the circulation may be preferable, especially when membrane barriers will prevent liposomes from reaching target sites.

In the blood, drugs are released from liposomes much more rapidly than expected from normal solute diffusion

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Gregory Gregoriadis is at the MRC's Clinical Research Centre, Harrow, Middlesex.

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through the bilayers (see reviews). This is attributed to the massive transfer of the liposomal component lecithin to the plasma high density lipoproteins (HDL) in turn leading to the loss of the carrier's integrity and ability to retain solutes (Krupp *et al. Biochem. biophys. Res. Commun.* **72**, 1251; 1976; Scherphof *et al. Biochim. biophys. Acta* **542**, 296; 1978; Chobanian *et al. Biochemistry* **18**, 180; 1979). It now seems that the plasma-induced structural damage to liposomes can be exploited to 'instruct' them either to retain entrapped drugs or release them at predetermined rates. Unilamellar or multilamellar liposomes can be made more stable in the circulation by incorporating cholesterol. Through modulation of phospholipid loss to plasma HDL (Kirby *et al., FEBS Lett.* **111**, 324; 1980), cholesterol controls bilayer permeability to solutes in the blood of injected animals in inverse proportion to its concentration (Gregoriadis & Davis *Biochem. biophys. Res. Commun.* **89**, 1287; 1979; Kirby *et al. Biochem. J.* **185**, 591; 1980). Sphingomyelin also has a similar effect *in vitro* (Finkelstein & Weissmann *Biochim. biophys. Acta* **587**, 202; 1979) although not necessarily by the same mechanism. Liposomes designed to retain their full drug load are expected to minimise the systemic toxicity of such drugs as the antimonials and metal chelators being tested in the intravenous treatment of experimental visceral leishmaniasis (Black *et al. Trans. Roy. Soc. Trop. Med. Hyg.* **71**, 550; 1977; New *et al. Nature* **272**, 55; 1978; Alving *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 2959; 1978) and iron loading (Guilmette *et al. Life Sci.* **22**, 313; 1978; Young *et al. Br. J. Haematol,* **41**, 357; 1979) respectively.

On the other hand, controlled release of drugs should promote their access to cells (tumours, for example) with which liposomes may not be able to interact directly (Rustum *et al. Cancer Res.* **39**, 1390; 1979). However, toxicity to normal tissues equally accessible to slowly diffusing drugs cannot be excluded. Therefore, for tissues which are amenable to hyperthermia, an alternative approach based on the property of liposomes to become leaky at the melting temperature of their phospholipid component (Yatvin *et al. Science* **202**, 1290; 1978; Scherphof *et al. Biochim. biophys. Acta* **556**, 196; 1979) may prove more useful. In this way, liposomal drugs delivered systemically can be released near and concentrate selectively in, appropriately preheated target areas such as tumours, infected or inflamed tissues (Weinstein *et al. Science* **204**, 188; 1979).

Whether systemic drug release or transport intact to target cells is required, the need for liposomes to persist in the blood for some time seems to rule out the use of the rapidly removed large liposomes (which may nonetheless be preferable for drug transport to the fixed macrophages or for capture in lung capillaries) and to favour the small unilamellar version. This, when uniformly sized, exhibits a linear rate of clearance and a half life which can be reduced as appropriate by the incroporation of negative charge.

Liposomes may also be introduced into the body other than through the circulation, and here the characteristics of the route of entry also influence the fate and efficiency of action of the liposomal drugs.

For instance, in arthritic rabbits injected intra-articularly with cortisol palmitate anchored on the lipid framework of liposomes, the degree and duration of the anti-inflammatory activity of the steroid is greatest in the initial acute phase of inflammation and is probably related to the phagocytic activity of the synovium (at later phases there are marked changes in its content of cell types) (Shaw *et al. Br. J. exp. Path.* **60**, 142; 1979). Interestingly, a similar beneficial effect of the liposomal steroid has been demonstrated in a few patients with rheumatoid arthritis (de Silva *et al. Lancet* **i**, 1320; 1979).

In another topical use potentially applicable to the chemotherapy of pulmonary metastases, an anti-tumour agent entrapped in positively charged liposomes and administered through the intratracheal route acted in the lung without adverse side effects in other tissues (McCullough & Juliano *J. natn. Cancer Inst.* **63**, 727; 1979).

After injection into tissue (which is of a wider therapeutic scope), liposomes can either disintegrate locally or migrate into the lymph nodes draining the injected tissue (Segal *et al. Clin. Sci. molec. Med.* **49**, 99; 1975). It has now been shown (Osborne *et al. Int. J. Nucl. Med. Biol.* **6**, 75; 1979) that localisation in the primary and secondary regional lymph nodes is enhanced dramatically when small uncharged liposomes are used. In addition to its obvious usefulness in the detection and treatment of tumours of the lymphatic system lymph node localisation of liposomes may also be the cause of their immunopotentiating property (Allison & Gregoriadis *Nature* **252**, 252; 1974; Morein *et al. Nature* **276**, 715; 1978; van Rooijen & van Nieuwmegen *Immun. Commun.* **8**, 381; 1979). This immunopotentiating effect has already shown promise in vaccine development (for example in conjunction with hepatitis B virus surface antigen for a vaccine against hepatitis, Manesis *et al. FEBS Lett.* **102**, 107; 1979).

It has become apparent that ways of controlling the fate of locally applied liposomes can extend beyond simple manipulation of their size and charge. A related development is the finding (Mauk *et al. Science* **207**, 309; 1980) that the rate of disintegration of liposomes in injected tissues can be varied widely by the incorporation of various sugars and amino sugar derivatives of cholesterol into the lipid structure. These derivatives seem to mediate binding of the associated carrier to the intercellular or cell surface components which, in turn, controls its endocytosis. Such modifications could prove useful in cases where interaction of liposomes with and uptake by cells (for example, gut cells after oral administration) is at present poor or non-existent. □

Io: the electrified satellite

from Garry E. Hunt

OF the 14 Jovian satellites, Io, one of the four planet-sized Galilean moons, presents the greatest anomaly, not only of the Galilean satellites but also among all the other bodies in the Solar System. During the recent Voyager encounters spectacular eruptions were seen on Io which have been initially interpreted as evidence for extensive volcanic activity (Smith *et al. Science* **204**, 951; 1979; Smith *et al. Science* **206**, 927; 1979). But is this the only possible interpretation? Gold (*Science* **206**, 1071; 1979) suggests an alternative explanation of these dramatic observations. He suggests that the passage of Io through the powerful magnetic field of Jupiter induces electric currents in Io's crust which heat up areas of the surface and cause the ejection of volcanic-like plumes of gaseous and solid material. Is this hypothesis possible?

There is no doubt that the eruptions on Io were probably the least expected, and most exciting, results of the Voyager mission. Certainly before the mission, the generally accepted view was that the surface of Io would be heavily cratered rather like the Moon. However Peale *et al.* (*Science* **203**, 892; 1979) had pointed out that since Io is subjected to resonant gravitational forces exerted by its sister moon Europa, its orbit would be distorted from its approximately circular path. Consequently, Io would be affected also by Jupiter's powerful gravitational field so that this repeated tidal flexing would create an enormous amount of frictional heat in the satellite's interior. This heat would ultimately have to be dissipated through the surface of Io. Would this necessarily result in widespread volcanism?

Smith *et al.* (*op. cit.*) observed eight active plumes on the first Voyager encounter, of which six were still active at the time of the second flyby three months later. In both cases, material was seen to reach altitudes of 70-280 km at ballistic speeds of up to 1 km s^{-1} (Cook *et al. Nature* **280**, 743; 1979). Interestingly, all except one of these plumes lie within ±30° of the equator (Strom *et al. Nature* **280**, 733; 1979). The eruption sites appear as regions with a central dark or black irregular to

Garry Hunt is the director of the Laboratory for Planetary Atmospheres, Department of Physics and Astronomy, University College London.

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circular spot that generally appears to have radiating dark, jet-like streaks. In many ways, the vents mapped from the Voyager imaging data appear to resemble closely terrestrial calderas or pit craters (Masursky *et al. Nature* **280**, 725; 1979). The floors of these features range from externally shallow to about 4 km in depth. However at least 300 caldera-like features have been detected randomly distributed over Io's surface and not therefore controlled by strongly patterned convection cells.

The south polar region is certainly important in these discussions. McCauley *et al.* (*Nature* **280**, 736; 1979) have observed white or bluish patches along scarps and faults, particularly in this region. Such features are diffuse, sometimes variable and obscuring the surface, suggesting that they may be cloud produced by gas leaking out of the interior of the satellite and condensing into some form of snow. Certainly, Pearl *et al.* (*Nature* **280**, 755; 1979) have detected a tenuous atmosphere of sulphur dioxide, while several observers (Fanale *et al. Nature* **280**, 761; 1979; Nash & Nelson *Nature* **280**, 763; 1979; and Smythe *et al. Nature* **280**, 766; 1979) indicate the presence of this material on the surface of Io. The surface is then probably a mixture of sulphur and sulphur dioxide. So there may be reservoirs of liquid sulphur dioxide on Io analogous to the lakes and oceans of the Earth. How therefore can we account for the plume features?

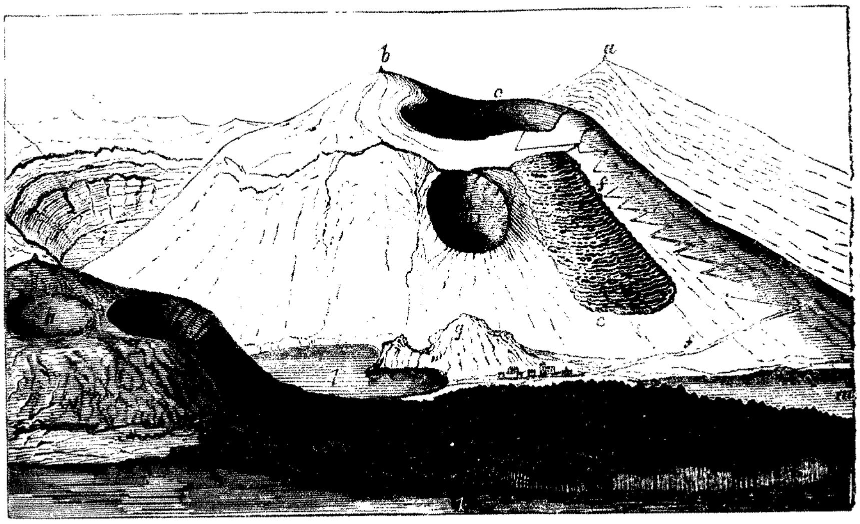
In certain locations, the observations suggest that ice clouds are issuing from fractures in Io's crust, and there is no doubt that liquid sulphur dioxide would escape to the surface along a fault or at the base of a scarp. Certainly, as it reaches the surface the sudden reduction in pressure to below the critical point would cause the liquid to explode into an ice fog that would spread out and then ultimately fall back to the surface again. The process may also erode material along the scarps as well, as McCauley *et al.* (*op. cit.*) suggest. Could this procedure account for the larger plumes that have been observed?

Smith *et al.* (*op. cit.*) suggest that this is the case, with the liquid sulphur dioxide causing violent reactions. This model requires large segregated pools of sulphur below the satellite's surface, and there are certainly some volcanic-like flows that may be of pure sulphur as Sagan (*Nature* **280**, 750; 1979) suggests.

However, an alternative view is taken by Carr *et al.* (*Nature* **280**, 729; 1979) who suggest that the volcanism is not primarily driven by the tidal forces, but by a mechanism in which the sulphur-enriched silicate magma erupts through a silicate crust enriched in sulphur. The variations in the physics and chemistry of the magma chambers may then account for the different types of eruption and therefore the different features on Io. Certainly it is difficult to imagine that volcanism on Io has continued at the same rate throughout the history which is implied by the tidal heating model. The other volcanic bodies, Mars, Venus, Moon and Earth, have certainly had variable periods of volcanism. So why should Io be different?

Gold (*op. cit.*) however, suggests that all these geologically-based interpretations are unsatisfactory. While there is no disputing the altitude and speed of the material in these plumes, he suggests that such behaviour is unlikely for sulphur compounds under volcanic conditions. He indicates that for such material to reach the observed speed of up to 1 km s^{-1}, it would have to be heated to about 6,000 K. Certainly the temperatures that can be expected in any volcanic region are likely to be below 1,500 K for nearly all rock-forming minerals. Furthermore, volcanic activity is supposedly more extensive on Io than on the Earth, so that the internal heating of Io would therefore be greater. But Gold calculates an Ionian heat flow of only 46 erg cm^{-2} s^{-1} from the data of Peale *et al* (*op. cit.*) compared with 65 erg cm^{-2} s^{-1} for the Earth.

With these inconsistencies, further examination of the Io eruptions is required. Gold points out that the satellite resides in the intense Jovian magnetosphere so that it will be electrically heated. Certainly Io, with a density of 3.5 g cm^{-3}, is composed of materials that conduct electricity and currents will flow through it as the satellite moves through the Jovian magnetic field. If this electric arc interpretation is correct, then the outbursts would repeat in a systematic way, controlled by the repetition cycle of the external field configuration. Local hot spots would be created, which is consistent with the observations by Hanel *et al.* (*Science* **204**, 972; 1979). The strong concentration of the majority of the plumes around the equatorial region indicates that this mechanism is appropriate in that region. However, in other locations, such as those of the poles, sulphur dioxide venting may be more appropriate. The eruption characteristics of Io may therefore be a complex mixture of volcanism and the results of the special electrical environment in which the satellite resides. Whatever the explanation there is little doubt that Io is in a hyperactive state, with the youngest surface currently known in the Solar System. □



100 years ago

Lipari is about ten and a half square miles in area. The highest point is 1,978 feet above the sea. Everywhere the island betrays its volcanic origin. Tuff, pumice, liparite (quartz-trachyte), and obsidian, are constantly met with; at San Calogero a hot spring (198°F.) pours forth water charged with carbonic acid and sulphuretted hydrogen, while the Bagno Secco discharges steam, sulphurous acid, and (it is said, but I think the statement requires confirmation) hydrochloric acid.

Vulcano is undoubtedly the most interesting member of the group from a volcanic point of view. It lies between four and five miles to the south of Lipari, and contains a semi-active crater, which, as regards its usual dynamic activity, occupies a mean position between Vesuvius in its present state of action, and an actual solfatara like that of Puzzuoli. Sulphur, alum, and boracic acid are the substances procured from the crater. We noticed also sublimates of sulphide of arsenic, and salts of copper were found in association with some of the aluminous incrustation; also chloride of ammonium. I have been assured by two eye-witnesses that blue and green flames sometimes issue from clefts in the bottom of the crater.

Prof. A. Cossa (*Gazetta Chimica Italiana*, 1878, p. 235-246) has pointed out that Vulcano furnishes the richest supply known of caesium and rubidium. The Faraglioni, also called *rocca dell' alume*, is a trachytic mass much decomposed by sulphurous and sulphuric acids; potassium-alum is found in its cavities, associated with the sulphates of aluminium and calcium, with chloride of ammonium, and with the alums of thallium, caesium, and rubidium. The most complex mixture of volcanic products hitherto found was discovered by Cossa on the edges of a small fumerole at the bottom of the crater of Vulcano. It was found to consist of the sulphides of arsenic and selenium, chloride of ammonium, boracic acid, sulphate of lithium, together with caesium- and thallium-alums, and traces of the alums of potassium and rubidium. From *Nature* **21**, 26 Feb., 401; 1880.

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REVIEW ARTICLE

Nuclear weapons and power-reactor plutonium

Amory B. Lovins

Consultant Physicist, c/o Friends of the Earth Ltd, 9 Poland Street, London W1V 3DG, UK

With modest design sophistication, high-burn-up plutonium from power reactors can produce powerful and predictable nuclear explosions. There is no way to 'denature' plutonium. Power reactors are not implausible but rather attractive as military production reactors. Current promotion of quasi-civilian nuclear facilities rests, dangerously, on contrary assumptions.

NUCLEAR policy, especially in Europe, has often been justified by the belief[1-10] that for making nuclear bombs, 'reactor-grade' plutonium produced by the normal operation of uranium-fuelled power reactors is necessarily much inferior to specially made 'weapons-grade' Pu: so inferior in explosive power or predictability that its potential use by amateurs is not a serious problem and that governments would instead make the higher-performance weapons-grade Pu in special production reactors.

Although that belief is false it was vigorously asserted during 1978–79 by responsible Ministers or by Prime Ministers in at least three high-technology nations. This was apparently because some of their nuclear experts did not know differently, and rejected contrary official US statements as being exaggerated or even politically motivated; or because those experts who did know were not asked; or because correct technical advice was lost, oversimplified, or garbled in transmission through advisors who did not understand the physics. Here I attempt to clarify the properties and performance of various grades of Pu, outlining the physical logic explicitly enough to ensure understanding although discreetly so as not to help the malicious: certain details and technical references have, therefore, been omitted and some calculations treated in conclusory fashion.

The possible military utility of power-reactor Pu has caused widespread professional confusion since 1946. Although some leading scientists appreciated even then[1,11] that it was useable in bombs or might become so, the contrary assumption (admittedly hedged) was made in the Acheson-Lilienthal report[12,13]. This recommended that certain nuclear activities could be classified as 'safe' because the Pu they produced could not be converted without timely warning into weapons-useable form, and that these activities could thus be carried out by nations if under strict and enforceable international controls. With the radical 1953 Atoms for Peace initiative, the report's finding that international inspections and treaties could not stop proliferation was forgotten, and US nuclear knowledge and materials were distributed worldwide with increasing enthusiasm and decreasing care[14]—apparently on the assumption that power-reactor Pu was, or could be made, unsuitable for use in bombs ('denatured'), despite the US Atomic Energy Commission's refutation of this notion[15] in 1952. With this in many signatories' minds, the Non-Proliferation Treaty (NPT) was negotiated in 1967: an important fact to recall when construing the NPT today, even though its text and the International Atomic Energy Agency (IAEA) do not distinguish in quality between Pu made in power or in military production reactors.

Beginning publicly in the early 1970s in the US (earlier in the Soviet Union and France[16]), the assumption that power-reactor Pu was unsuitable for bombs was questioned with increasing force[17,18]. By 1974, authoritative reports[19,20] had concluded that reactor-grade Pu could be used even by amateurs to make effective fission bombs, albeit of somewhat reduced and uncertain yield[21-25]. Further analyses during 1976–77, mainly at the US weapons laboratories, revealed a still wider spectrum of technical possibilities, and in 1977 the US announced that it had successfully tested a bomb made from reactor-grade Pu[26,27]. Yet the earlier finding that this material, though useable, was inferior if used in relatively crude bomb designs was widely and wrongly supposed to apply to all designs. In particular, reactor-grade Pu was alleged to be inherently:

—far more hazardous than weapons-grade Pu to people dealing with it; or

—far more likely to cause unintentional explosions; or

—incapable of exploding violently enough to do much damage, or, at worst, to accomplish most military aims; or

—too unpredictable in explosive yield to be acceptable to its users.

Each of these assumptions contains, in certain circumstances, some truth; but each is generally, or can by plausible countermeasures be rendered, false. Their implication that reactor-grade Pu is not very dangerous, or unlikely to be attractive to governments, is wishful thinking, and causes the proliferation risks of 'civil' nuclear activities to be gravely underestimated.

In recent years, advocates of commercial Pu use, in referring to the bomb-making that might also result, have had to retreat "from the original concept of denaturing to the notion of making do with less than optimal material"[11]; yet the earlier myth lingeringly distorts policy[28]. To decide responsibly about nuclear power and nuclear fuel reprocessing, we must know exactly what "less than optimal" means, and hence must cautiously review published physical principles. The only thing more dangerous than discussing this subject is not discussing it; the lesson of Atoms for Peace may yet be that with Pu, we must get our assessments right the first time.

Plutonium production

All nuclear reactors fuelled with uranium[29] (notably the 0.7%-naturally-abundant species ^{235}U) produce ^{239}Pu by neutron absorption in fertile ^{238}U. Some of the ^{239}Pu formed is fissioned; an increasing fraction absorbs successively more neutrons to form higher Pu isotopes, chiefly $^{240-242}$Pu, and transplutonic elements[30]; and some of the resulting $^{239-242}$Pu (plus traces of ^{238}Pu and related species) survives until the fuel is discharged. Depending[19,24,25] on the type and detailed design of reactor, the composition of initial fuel, and the manner of operation, especially the burn-up (extent of exposure of the fuel to neutrons), the net Pu production of a 1-GWe power reactor is typically several hundred kg yr^{-1}, for example ~210–240 kg yr^{-1} for a light-water reactor (LWR), the dominant commercial type.

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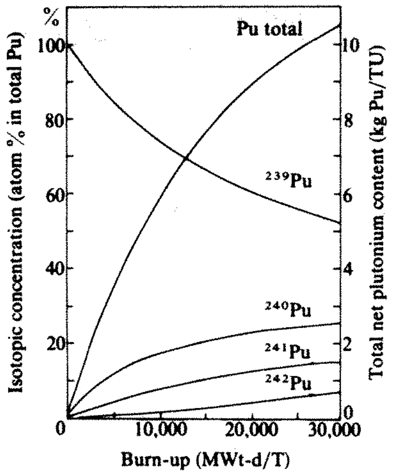

Fig. 1 Typical total amount and isotopic composition of plutonium in discharged fuel as a function of burn-up in a light-water reactor[32]. The exact values depend in detail on reactor design and operation and on initial fuel composition. Higher isotopes of plutonium and transplutonics become more abundant in repeatedly recycled fuel.

For a given reactor, the isotopic composition of the discharged Pu depends predictably on burn-up. If each metric ton of uranium (TU) yields only a few gigawatt-days' thermal energy (GWt-dT), neutron capture is so limited that nearly pure ^{239}Pu—'weapons-grade' Pu—is produced. It contains no more[1] than 7%[24] or 8%[11] ^{240}Pu, typically[31] around 6%; perhaps 0.5% ^{241}Pu; negligible ^{242}Pu and ^{238}Pu.

Higher burn-up, whether through leaving the fuel in the reactor longer or exposing it to a more intense neutron flux, produces a larger proportion of the higher Pu isotopes (Fig. 1). Low-enriched uranium fuel (the usual kind) left for the full 3–4 yr in a reliably operating LWR is usually designed for a nominal burn-up of 27–33 GWt-dT. The latter corresponds[33] to discharge Pu containing ~58% ^{239} Pu and 11% ^{241}Pu (both fissile) plus 25% ^{240}Pu, 4% ^{242}Pu, and 2% ^{238}Pu. This composition is broadly similar for other thermal or fast reactor types, except for graphite-moderated reactors[34] ($^{240+242}$Pu ~ 20%) and fast breeder radial blankets[30] (~ 4%). In some circumstances the $^{240+242}$Pu fraction can rise from a nominal ~29% to as much as 49% (34% ^{240}Pu, 15% ^{242}Pu)[33] in equilibrium LWR Pu recycle fuel; this is unusually high and approximates a practical limit.

Power reactors also can, and often do, discharge fuel short of its full design burn-up. Mature US LWR cores routinely attain well below design burn-up[35]. Real or simulated malfunctions, such as leaky fuel cladding, may lead the operator to discharge fuel at very low burn-up[1]. Alternatively, manipulation of fuel rods can produce a core with high average burn-up but containing some rods of much lower burn-up. 'On-load-refuelling' reactors such as Magnox and CANDU are suitable for clandestine introduction, brief exposure, and removal of small but adequate amounts of fertile material in a few fuel channels. Such methods are not necessarily easy to detect even if an inspector were continuously present, and would probably not be detected at all by present international safeguards[36].

Because burn-up, and hence isotopic composition of discharge Pu, can vary enormously between and within reactors and with time, 'reactor-grade Pu' is not a well-defined term. Weapons-grade Pu, which is fairly well-defined (supra), can be readily produced in any power reactor without necessarily and significantly decreasing efficiency, increasing costs, or being detected. But though that option is always open, we assume here that it is not—that power reactors will be used to produce only high-burn-up Pu. We also assume that 'reactor-grade Pu' implies a $^{240+242}$Pu content of ~30%; higher, even arbitrarily higher, even-isotope content will not affect the argument, while a lower content would strengthen it. On these conservative assumptions, the Pu discharged from power reactors will be shown to have major military potential.

Plutonium properties

Relevant nuclear properties of the common Pu isotopes are summarised in Table 1. The values given for m_c are not the quantities needed to make a bomb, as neutron reflection and implosion can reduce critical mass by a large factor. This factor has been officially stated to be ~ 5, consistent with the US[39] and IAEA requirement of strict physical security measures for quantities of Pu $\geqslant$ 2 kg (independent of isotopic composition). Published data suggest, however, that with sophisticated design the factor may be > 5. For example, in its densest (α-phase) allotropic form, ^{239}Pu at normal density in a thick Be reflector has a reported critical mass as small as $m_c/4$, and large reflector savings are also obtainable with nonmoderating (and partly fast-fissionable) reflectors such as ^{238}U. Further, if the core and reflector are equally compressible and if the ratio of reflector thickness to core radius remains constant during compression, critical mass varies as the inverse square of core density. Densities 'several times' normal are said to be attained in military

Table 1 Some properties of plutonium[31,34,37,38]

Isotope	Decay properties: Half life (yr)	Decay properties: Main emission (MeV)	Specific activity (Ci g^{-1})	Heat (W kg^{-1})	Fast-fission: m_c* (kg)	Fast-fission: $\bar{\nu}$†	Fast-fission: σ_f‡ (barn)	Spontaneous fission: Rate (n g^{-1} s^{-1})	Spontaneous fission: $\bar{\nu}_{SF}$§
^{238}Pu	86.4	5.5 α	17.4	567	9	~3‖	2.5‖	2,600	2.3
^{239}Pu	24,390	5.2 α	0.061	1.9	10	3.1	1.9	0.03	2.9
^{240}Pu	6,600	5.2 α	0.23	7.0	40	3.4	1.3	1,020	2.2
^{241}Pu	13.2	0.021 β	112	4.5	12	3.2	1.8	—	—
^{242}Pu	387,000	4.9 α	0.0038	0.1	90¶	3.3	1.2	1,670	2.3
^{241}Am #	433	5.5 α	3.43	106	114**			0.623	3.1

* Approximate bare-sphere prompt-critical mass at normal density; Pu is α-phase ($\rho = 19.8$ g cm^{-3}). For comparison, m_c for 93.5% ^{235}U ($\rho = 18.8$ g cm^{-3}) is 49 kg ^{235}U.

† Approximate gross neutron yield per fast fission (Argonne 16-group).

‡ Approximate fast-fission cross-section (Argonne 16-group). (The capture-to-fission ratios are also important, and tend to be high for even Pu isotopes.)

§ Approximate neutron yield per spontaneous fission.

‖ Values cited by De Volpi[37], who gives the ^{238}Pu m_c as 7 kg (unpublished); the best value calculated for α-phase, however, is the $m_c \sim 9.2$ kg shown here (R. W. Selden, personal communication).

¶ Best Livermore estimate from the range 75–100 kg (R. W. Selden, personal communication). De Volpi[37] assumes ~95 kg (or 118 kg in Appendix H), but used $\geqslant$156 kg in earlier publications[45,46]. Calculated m_c is probably not finite for ^{242}Pu^{10}B$_2$, owing to threshold effects, but is ~350 kg for ^{240}Pu^{10}B$_2$ at $\rho = 11.5$ g cm^{-3} (R. W. Selden, personal communication).

# Daughter of ^{241}Pu, important (like the trace isotope ^{236}Pu) mainly for its γ emissions.

** Approximate estimate by Clayton[38] for $\rho = 11.7$ g cm^{-3}, implying reactivity comparable with ^{240}Pu.

bombs[40], and by means described in the open literature a slightly subcritical mass of α-phase ^{239}Pu in a U reflector can be compressed to 1.85 times its initial density, corresponding to a reactivity increase of ~3.4 times. The same method can yield even higher compressions. Although high compression is inconsistent with simultaneous high reflection, it may be possible to achieve significant supercriticality with initial ^{239}Pu masses under 2 kg. Regardless of the exact figure, the IAEA's 8-kg 'significant quantity' design basis for detecting diversions[36] seems far too high.

Of the Pu isotopes in Table 1, only ^{239}Pu and ^{241}Pu are fissile, that is, fissionable by thermal (slow) neutrons. All Pu isotopes, however, are fissionable by the fast neutrons in a bomb: indeed, at energies > 1 MeV (69% of fission neutrons) the fission cross-section of ^{240}Pu is less than that of ^{239}Pu by a margin of less than 20%, so the amount of ^{239}Pu required to form a normal-density critical mass is remarkably insensitive to $^{240+242}$Pu content (Table 2). Published neutron transport calculations confirm that the even isotopes produce only minor changes in reactivity, neutron spectrum, and mean prompt-neutron lifetime. So reactive is ^{240}Pu that changing the $^{240+242}$Pu content from 6 to 30% increases m_c only from ~11 to ~13 kg[31]. No known fast-neutron absorber can make Pu of any practical composition incapable of forming a prompt-critical mass[31,42]: calculations[42] suggest that substituting the best fast-neutron absorber known, ^{10}B, for oxygen in reactor-grade crystal-density PuO_2 ($m_c \sim$ 35 kg) would increase m_c only to ~70 kg.

Fast-fission properties of Pu, then, are only slightly affected by ordinary changes in isotopic composition. The neutron background is, however, modestly affected. Spontaneous-fission neutrons (Table 1) provide typically[34] of the order of 100 n g^{-1} s^{-1} in weapons-grade Pu, about 500 in reactor-grade Pu. A further contribution (at lower neutron energies) comes from (α, n) reactions with light-element impurities. In Pu metal this is not an important neutron source[34], but it can more than double the neutron background in PuO_2, as 1 Ci of α-emitter bombarding oxygen produces of the order of 5,000 n s^{-1}.

Neutron background from all sources is significant for weapons physics (infra) and makes reactor-grade Pu give somewhat higher radiation doses than weapons-grade Pu. For long-term commercial handling within the normal canons of health physics, shielding would be required. For clandestine weapons manufacture it would not, because the published total dose rates (mainly γ and X rays) are relatively modest—of the order of rem h^{-1} at the surface of a 1-kg fresh recycled PuO_2 sphere, < 1 mrem h^{-1} at 1 m. Even under extreme assumptions (18½% ^{238}Pu, 30% $^{240+242}$Pu, 10-kg PuO_2 sphere with subcritical multiplication included), neutron dose rates (60 mrem h^{-1} at 1 m) "do not provide an effective deterrent"[44]. It is obviously not correct that "slight mistakes in the knowledge of exact [isotopic] composition .... may well pose extreme radiological hazards"[43], given normal precautions against inadvertent criticality.

The specific activity of reactor-grade Pu is typically[42] ~10 Ci g^{-1} as against 3 Ci g^{-1} for weapons-grade Pu, the neutron background (for metal) and the inhalation toxicity ~5 times higher, and the heat production ~10 W kg^{-1} (about the same as for 1-yr-old spent LWR fuel) as against 3 W kg^{-1}. Only a difference of orders of magnitude in these quantities—the extent by which they all exceed the corresponding values for ^{235}U—would reflect differences in ease of handling that would be important in designing new bomb-making facilities[42].

'Thermal denaturing'[44–46] of reactor-grade Pu was recently proposed by A. K. Williams *et al.* (unpublished Allied-General Nuclear Services) and A. De Volpi[37]. The ^{238}Pu content would be increased from its normal 1–2% (nearly 5% in some recycle Pu[33]) to 10–15%, perhaps nearly 20%, chiefly by recycling ^{237}Np (itself a bomb material with $m_c \sim$ 75–105 kg) with ^{232}Th rather than ^{238}U diluent[29]. The ^{238}Pu would increase heat production to 6–9 times normal. Some calculations[44] based on cores of high mass and high volume-to-surface ratio, in direct contact with non-thermally-stable explosives, suggest serious design problems. But realistic choices of geometry and materials can readily overcome these problems. Cooling does not even become an interesting design problem until the ^{238}Pu content approaches that of a thermoelectric heat source, and a much lower content, well over 20%, does not provide an effective deterrent even to most amateurs, though it would make safe and economic fuel-cycle operations hard to envisage[47]. Only in quite incompetent hands could the extra heat pose a danger of unplanned detonation.

Table 2 Critical mass of plutonium spheres in a ~10-cm natural uranium reflector as a function of plutonium isotopic composition[41]*.

$^{240+242}$Pu (atom %)	^{239}Pu in critical mass (kg)	Total Pu in critical mass (kg)
0	4.4	4.4
10	4.5	5.0
20	4.5	5.6
30	4.6	6.7
40	4.7	7.8
50	4.8	9.6

* Assuming ^{240}Pu/^{242}Pu ratio similar to that shown in Fig. 1.

A second 'denaturing' proposal by De Volpi[37] would combine ^{238}Pu and ^{240}Pu with extra ^{242}Pu, which is a diluent in fast spectra and something of a neutron poison in thermal spectra[48]. Pathologically high ^{242}Pu content would increase m_c, though not by the ~30 times claimed by Olds[45] (compare Table 1); as all Pu isotopes have "reasonably small critical masses, this concept [of denaturing by dilution] is not applicable to plutonium"[44]. De Volpi states[46] that fuels made mostly of ^{242}Pu and with a fissile fraction as low as 18% are useable even in present fast reactors, but he does not analyse performance or economics, which apparently offer serious difficulties[48], and his analysis of weapons physics seems deeply flawed.

Synergisms are negligible, so the effect of each even Pu isotope can be dealt with separately: thermal 'denaturing' with ^{238}Pu above and neutronic 'denaturing' with all three isotopes below. Practicable dilution with ^{242}Pu cannot alter design constraints enough to affect the conclusions below.

Finally, nonmetallic forms of Pu must be considered. Reactor-grade Pu can be used directly in bombs without reduction to metal (though metal generally gives better performance). The oxygen in PuO_2 dilutes the Pu and lengthens the 'generation time' between fissions, reducing the yield, but it also slightly compensates by moderating the neutrons to lower energies where the fission cross-sections are higher. The dilution effect predominates and roughly doubles[42] the δ-phase metallic m_c. It also alters other design parameters. Nonetheless, the oxygen is not a neutron poison and does not prevent attainment of "large reactivity coefficients and short neutron life times"[18] even in the much less reactive $^{235}UO_2$. Indeed, the low initial density ($\rho \sim$ 2.3 g cm^{-3} uncompacted, 4–5 g cm^{-3} moderately compacted) of PuO_2 powder relative to its sintered ($\leqslant$ 11.2) or crystal (11.5) density, and its relative compressibility at crystal density, permit crude designs with a generous safety margin of initial subcriticality to achieve high supercriticality after implosion. It is, therefore, not surprising that PuO_2 has been uniformly agreed to be directly useable in formidable explosives[18,19,24,25,41]. A bomb made directly even from fresh LMFBR mixed-oxide fuel (15–25% Pu) is theoretically possible[49], though unwieldy.

Weapons physics and reactor-grade plutonium

A nuclear explosion results from a divergent chain reaction in a prompt-supercritical mass: that is, one which can support a chain reaction by virtue only of the prompt neutrons released immediately by fission. The fission neutrons in a bomb are fast, with energies in the megavolt range and velocities of the order of 1–2 × 10^9 cm s^{-1}. The fissionable material must be of sufficient size and density that a neutron born within it is likely to cause a further fission within it. The critical masses discussed above imply that the mean distance between fissions is of the order of centimetres, hence that the mean time between fissions is of the order of 10^{-8} s. Roughly "40–50 generations of fissions are required to build up a fast chain reaction to an explosive level"[41]: $e^{40} = 2.4 \times 10^{17}$ fissions would release 1.6×10^3 kcal, equal to the nominal energy release from ~1.6 kg of high explosive and thus approaching the energy density needed to disassemble rapidly a Pu core of nominal size. Only the last few generations in this exponential process provide appreciable energy yield. That energy builds up, in a few hundredths of a microsecond, temperatures of several hundred million °C and pressures of the order of 10^8 bar, causing the core to expand with a velocity of the order of 10^8 cm s^{-1}. Expansion from the initial radius by a factor roughly equal to the sixth root of the number of critical masses present, that is, by ~1 cm, makes the mass subcritical and quenches the chain reaction. Once the explosion begins, then, only a few more generations of fission, producing most of the yield, are possible.

The designer seeks to assemble as supercritical a mass as possible (by 'inserting reactivity') as quickly as possible, and to inject neutrons to initiate the chain reaction at the optimal moment during that assembly—before the instant of maximum supercriticality, so that the bomb's tendency to fly apart is partly countered by the continuing forces of convergent assembly. The yield of the nuclear explosion depends strongly on the degree of supercriticality achieved when the chain reaction is initiated. If the neutron background is so large that preinitiation, before the optimal moment, is likely, then the yield varies[18] as (rate of reactivity insertion)$^{-1-1.5}$.

Preinitiation reduces yield according to the Poisson statistics of neutron background, and can be counteracted by faster assembly. For a constant level of maximum reactivity insertion, the probability of avoiding preinitiation varies as 1/exp (neutron source strength × assembly time).

Preinitiation is a problem faced by any nuclear weapons designer using any fissionable material[31]. With ^{235}U (spontaneous fission rate 8×10^{-4} n g^{-1} s^{-1}), it is a mild problem, so assembly by the relatively slow 'gun' method at published rates <1 mm μs^{-1} (0.3 mm μs^{-1} in the Hiroshima bomb) suffices. But even weapons-grade Pu, with neutron background ≥60,000 times higher (just above ^{238}U), preinitiates unless assembled implosively by a convergent arrangement of chemical high explosives. The radial compression rate can then[32] exceed 2 mm μs^{-1}.

A reflected core of Pu metal, sufficiently subcritical that neutron multiplication can be neglected, will have a neutron background of the order of 0.5×10^6 n s^{-1} if of weapons grade, several million n s^{-1} if of reactor grade. The former figure implies a mean time between neutrons of a few microseconds—half an order of magnitude longer than the duration of the fission chain. But the latter figure implies a mean time between neutrons that is short compared with the time required to complete the assembly of a highly supercritical mass, unless that assembly is extremely rapid, implying very high shock velocities and compression. This can in fact be achieved:

> "Plutonium of any feasible grade (weapons or reactor) is unsuitable for the gun-assembled systems because the neutron background and relatively long assembly time introduces significant preinitiation probabilities. This is not necessarily true for implosion types"[34].

Preinitiation, then,

> "... does *not* necessarily make an explosive unreliable. Preinitiation *does* result in a statistical uncertainty in the yield ... [that] is statistically distributed between predictable upper and lower limits which are likely to be more than a factor of 10 apart. For a *well-understood design properly constructed*, however, the most probable yield range could be predicted within much closer limits"[24].
>
> "In implosion devices, preinitiation probabilities may be lowered by various design techniques. Even relatively low technology designs, notwithstanding the variability in yield, *can produce effective, highly powerful weapons*"[34].

This can be achieved[41] even with a neutron source strength of the order of 10^6 n s^{-1}. As was officially appreciated in 1944,

> "... [A] rough quantitative analysis of the assembly velocities attainable with very large charges of high explosive ... suggested that because of the strong focusing effect of the converging material, one could introduce a strong steady source of neutrons into the bomb (for example, by deliberately leaving the material in an impure state), and still beat the chain reaction and attain complete assembly".

Thus preinitiation "... *may mean a statistical uncertainty in the yield within a predictable range.* Increasing technological sophistication will reduce this uncertainty"[31].

Now, consider several several levels of "increasing technological sophistication" and their likely effects on the performance of implosion bombs made from reactor-grade Pu or other compositions[37] with high neutron source strength.

Performance as a function of design

Since it became public knowledge that construction of illicit nuclear weapons by non-state adversaries must be taken seriously[19], and that reactor-grade Pu is suitable for such bombs[17,18,24], the level of technology on which most discussions have centred can be described as 'crude amateur designs' in which the normal military design requirements for pure fissile material and for the symmetry, simultaneity, and speed of implosion are very considerably relaxed. The canonical description of such designs is that of Willrich and Taylor[19]:

> "Under conceivable circumstances, a few persons, possibly even one person working alone, who possessed ~10 kg of plutonium oxide and a substantial amount of chemical high explosive could, within several weeks [or perhaps less], design and build a crude fission bomb... [that] would have an excellent chance of exploding, ... probably ... with the power of at least 100 tons of chemical high explosives. This could be done using materials and equipment that could be purchased at a hardware store and from commercial suppliers of scientific equipment for student laboratories.... [It] might yield as much as 20 kilotons of explosive power—the equal of the Nagasaki A-bomb [though the probability of such a high yield is quite small]."

"... very sophisticated thermal-hydraulic and neutronic calculations"[43] would not be needed. Several suitably comprehensive literature searches have in fact been conducted by amateurs. The bomb could fit in a car[41].

Making an effective, transportable fission bomb is not a trivial task. Safely realising a paper design in properly working apparatus would require alertness to subtleties and care and skill in technical arts (which many criminal enterprises have shown). The necessary human resources will be assumed here to have been obtained. The skills required for both design and fabrication naturally increase with increasing sophistication and have been taken into account in the policy conclusions drawn here.

Several studies[32,43,50,51] have dealt quantitatively with the heterogeneous class of flexible and imprecise designs grouped here as Level One technology. Calculations confirm that in the absence of gross incompetence or malfunction of major components, Level One technology is extremely likely to yield >0.01 kton, likely to yield in the range 0.1–1 kton, able on occasion to yield several kton, and unlikely to yield 10–20 kton.

A second level of technology might be characterised as 'imitation Trinity designs' or as 1945-vintage US technology. It uses special high-explosive components and detonators (all of which are commercially available) in a straightforward geometry to compress an accurately made metallic Pu core. Given the materials and information that have become widely available since the 1940s, such a technology might be typical of a low-level national effort to produce a bomb that can be depended on to work well without previous testing (Trinity yielded 17 kton). It is not a clever technology (simpler means can produce better results) but is basic and reliable, and has been the subject of several amateur design exercises. Five distinguished nuclear weapons experts concluded that using weapons-grade Pu (or ^{233}U or highly enriched ^{235}U)

> "... it is possible to design low-technology devices that would reliably produce explosive yields up to the equivalent of 10 or 20 kilotonne of TNT. With reactor-grade plutonium it is possible to design low-technology devices with probable yields 3–10 times lower than those mentioned above (depending on the design), but yields in the kiloton range could be accomplished. Militarily useful weapons with *reliable* nuclear yields in the kiloton range can therefore be constructed using low technology and reactor-grade plutonium"[24].

(This is consistent with De Volpi's Table L–6 results[37], given realistic choices of parameters.) As for the resources required, "a small group of people, none of whom have ever had access to the classified literature", could get by with "modest machine-shop facilities that could be contracted for without arousing suspicion" and with "a fraction" (perhaps a small fraction) of a million dollars for open-market equipment[24].

Recently declassified documents dealing with 1945 US technology give[1]

> "... quite precise quantitative information ... covering some aspects of how the probability distribution of nuclear yields changes with the isotopic composition of the plutonium used. ... This information makes clear that with power reactor grade plutonium, an implosion weapon of even the simple kind first used by the US would reliably have yields between 1 and 20 kton"[52].

Preinitiation at the least favourable moment[50] in a Trinity-type bomb would still produce[1] reliable kiloton yields: the worst-case, minimum, 'fizzle' yield is still a "militarily useful"[31] ~1 kton. This behaviour is chiefly a function of assembly rate, not neutron background, because the least favourable moment cannot get any less favourable. The same conclusion would therefore hold[42] even with arbitrarily high neutron source strength, as from 'a large fraction' of ^{242}Pu (ref. 46) and high ^{238}Pu (ref. 37). Contrary to Meyer *et al.*[43], "slight mistakes in the knowledge of exact isotopic composition" could not "lead to neutralisation of the explosive design" and could indeed be readily accommodated by design.

Selden summarises[31]:

> "It is likely that a nuclear explosive designer would choose to minimise the ^{240}Pu concentration, given the choice. However, *an entirely credible national nuclear explosives capability could be constructed using only reactor grade plutonium.*"

Of course, the designer may choose to avoid the extra fiscal and political cost (if detected) of the *unambiguously military* dedicated facilities required[13] to make large amounts of the more conveniently useable weapons-grade Pu. Some governments may find advantages, notably civilian 'cover', in doing the best they can with their plentiful high-burn-up Pu instead. Recent classified analyses have therefore explored further levels of technological sophistication that can overcome the sub-optimal features of high-burn-up Pu and increase the ~1-kton *minimum* yield of Level Two technology.

Perhaps the most categorical statement of the results comes from a speech[14] by Commissioner Gilinsky (emphasis added):

> "... [S]o far as reactor-grade plutonium is concerned, the fact is that it is possible to use this material for nuclear warheads *at all levels of technical sophistication.* In other words, countries less advanced than the major industrial powers but, nevertheless, possessing nuclear power programs can make very respectable weapons. And, I might add, these are the very countries whose names turn up in every discussion of proliferation. Of course, when reactor grade plutonium is used there *may* be a penalty in performance that is considerable *or insignificant*, depending on the weapon design. But whatever we might once have thought, we now know that even simple [Level Two] designs, albeit with some uncertainties in yield, can serve as effective, highly powerful weapons—reliably in the kiloton range."

The italicised passages imply that further levels of technology exist with still higher yield and predictability than Level Two.

A third level of technology seeks to overcome preinitiation by extremely rapid assembly. The necessary rates of assembly can be calculated and seem to be readily attainable. One published configuration, for example, is stated to be able to compress a sizeable core at several times the 2 mm μs^{-1} radial rate mentioned earlier. Far higher rates are possible with this method, because in a convergent implosive system, shock pressures vary roughly as the inverse fourth power of radius and shock velocities as the inverse square of radius. Applying the same design to a small core can therefore achieve such high assembly velocities that the shock can traverse much of the core and insert very substantial reactivity before hydrodynamic disassembly forces can dominate. The probability of preinitiation then becomes small, the expected yield relatively large, and its dispersion small: in short, the performance penalty approaches the "insignificant"[14].

Calculations suggest that this Level Three technology is sufficient, using reactor-grade Pu, for almost any military objective, even with attempted 'denaturing'[37]. The method will readily occur to most governments and to many technically informed amateurs. The apparatus is not unduly difficult to achieve. It does not necessarily involve a higher technology than Level Two, and should be considered more a logical extension of Level One, a 'smart amateur design', than the exclusive province of governments. It does not necessarily require nuclear testing to ensure confidence in obtaining high yield, provided the maker is confident that the well-characterised non-nuclear components will function as designed. Non-nuclear test firings would, of course, increase this confidence.

Most military bombs are required to function normally in high neutron fluxes such as might arise from nearby nuclear explosions. Essentially the same design considerations apply as in the case of high internal neutron source strengths. It can be safely presumed that these problems are routinely overcome by various means, including thermonuclear techniques that could render the penalty from using reactor-grade Pu not only very small but nil, even at very high yields. This is a very high technology requiring elaborate theoretical, computational, and fabrication facilities, together with prior nuclear testing. But such sophistication is not required for governments or even for some subnational groups to extract from reactor-grade Pu, using Level Three technology, the kind of yield and predictability that the major nuclear powers would have found satisfactory for inclusion in their arsenals around the early 1960s.

Weapons effects and the implications of uncertain yield

Fissioning 1 kg of Pu yields (2.5×10^{24} fissions) × (~180 MeV of prompt energy per fission) $\simeq 7.3\times10^{13}$ J kg^{-1} or $\sim 17\times 10^{12}$ gcal kg^{-1} $\equiv$ 17 kton kg^{-1}. This 17-kton yield (Hiroshima was 13 kton, Nagasaki 22 kton) corresponds to the conversion of a mass defect of only 0.8 g. But unlike chemical high explosives, some of the yield of nuclear explosives appears not as blast or heat but as prompt electromagnetic radiation at many frequencies, especially γ rays, and as prompt neutrons. The smaller the nuclear yield, the more the effects of the nuclear explosion are dominated by prompt radiation.

For example, a 'crude amateur design' yielding only 0.1 kton—which corresponds to fissioning only 6 g (a nominal efficiency of the order of 10^{-3}) and converting a mass defect of 5 mg—typically produces[19,53] 500 rem of prompt γ dose (roughly the LD$_{50}$, or dose likely to kill half those exposed to it) at unshielded ranges up to 300 m, plus 500 rem of prompt neutrons (~LD$_{50}$) to ~450 m, plus 500 rem of fallout exposure (~LD$_{50}$) to 300–1,000 m for people lingering for an hour. Yet such a surface burst makes a crater only ~14 m in radius. Blast damage is also of shorter range than the prompt radiation—severe (overpressure to 10 lb in^{-2} = 0.68 bar) to 150 m and moderate (3 lb in^{-2} = 0.20 bar) to 300 m. The relatively limited blast damage, even at such low yield, however, could amplify the radiation effects by ~10^3 times if used to induce a 1% ground release from an operating 1-GWe LWR (16-GCi inventory), ~10–100 times more from a reprocessing plant.

Effects of larger yields are readily, if roughly, calculable from standard scaling laws[19,53]. For example, a yield of 1 kton, a practical minimum with Level Two technology (but three orders of magnitude larger than a World War II blockbuster), would devastate several km^2, with 500 rem prompt radiation beyond 700 m and with 500 rem fallout around 1–3 km. If a yield Y expected to be 8 kton is in fact only 1 kton, the blast area will diminish not by 7/8 but by 3/4 (as $Y^{2/3}$), and the lethally irradiated area only by half (as $Y^{1/3}$) (refs 1, 53). Such reductions would generally be smaller than the variability in effect expected from the varying circumstances of use; and except in special tactical-warfare conditions, the important thing is not whether the designer can predict the exact yield, but rather that the potential victim cannot.

Conclusions

The above discussion has sought to provide a discreet, selective, but adequate physical basis for understanding the scope for using reactor-grade Pu in fission bombs at some of the diverse levels of sophistication open to various potential users (the taxonomy given is not exhaustive). 'Denaturing' Pu by adding to it, singly or in combination, essentially inseparable[54] neutron-emitting diluents such as ^{238}Pu, ^{240}Pu, ^{242}Pu, or ^{252}Cf (ref. 55)—or indeed any other interfering material that cannot be readily removed, for example, by ion exchange—is "fallacious" and "not a valid concept"[31]. (Dilution with UO_2 or other materials requiring chemical or physical[56] separation is a valid cessed[19,25,34]—but does not solve the problem.) Taking all effects on weapons physics into account, a high ^{238,240,242}Pu content may reduce expected yield to a level that could devastate only a reduce expected yield to a level that could devastate only a modest portion of a city rather than all of it, and may make that yield much less predictable, if the bomb is crudely made. But these faults can be overcome by more clever design, without necessarily using high technology, and at Level Three this can be done by gifted amateurs. It is therefore incorrect to state categorically that bombs made from reactor-grade or deliberately 'denatured'[37] Pu are less effective, less powerful, or less reliable than those made from weapons-grade Pu. Whether these reservations hold, and whether by a meaningful margin, depend on the designer's intentions, skills, and resources, all of which may be unknowable. And the implication that the effects of even a crude, minimal 0.1–1 kton explosion would be tolerable for a free society is at best disingenuous[19–21,27].

The foregoing argument also implies that power reactors are not an implausible but are rather potentially a peculiarly convenient type of large-scale military Pu production reactor. This goes beyond the proposition that

> "... in situations of extreme tension states may turn to second or third best instruments to get their hands on weapons they regard as essential to their security. The point is that, with plutonium readily available, it may be turned to. And those groups within countries that want to go nuclear can pursue an ambiguous path of keeping their options open until the last minute under a commercial disguise"[57].

A government can either manipulate civil fuel cycles to produce substantial amounts of low-burn-up plutonium, or, especially using Level Three technology, use high-burn-up Pu in bombs with insignificant performance penalty. Regardless of possible technical measures[24,25,54,58,59], such Pu will become readily available in quantities of the order of 10^4–10^5 m_c yr^{-1}, and in extracted forms useable for weapons within hours or days, if plans proceed for commercial reprocessing, which is unsafeguardable both today[36] and in principle[14,59,60]. Making even high-burn-up Pu in domestic power reactors incurs no penalty in reactor efficiency or in equipment costs: a reactor-exporting country will gladly build the reactor, train the technicians, and pay for the whole package with generous export subsidies. If extracted, and using any of the numerous means of evading effective safeguards, the Pu discharged from a single large power reactor suffices for about 10^2 bombs per year, a large weapons programme, and there is the alternative of embezzling up to a few bombs' worth per reactor-year from the fuel cycle within its ~1% statistical 'noise', a form of theft that can be made undetectable in principle. The marginal time and money required to use civil reactors for military production are orders of magnitude less[27] than those needed for dedicated military facilities[11,61]. The extra facilities and staff required could be hidden within the civil programme: the "ideal place to hide a tree is in a forest"[61]. And perhaps the greatest attraction of producing military Pu in a power reactor is that it has a civilian 'cover' and thus—at least until regional rivals follow suit—an apparently zero political cost.

In short, the somewhat greater technical difficulty of using power-reactor Pu for effective military bombs—assuming the reactor is actually operated at high fuel burn-up—may be more than counterbalanced by the greater political and economic ease of obtaining that Pu. It "should not be lightly disdained in favour of purer material from dedicated facilities"[61].

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ARTICLES

Permian–Triassic continental reconstruction of the Gulf of Mexico–Caribbean area

Gary W. White

Department of Geological Sciences, State University of New York at Albany, Albany, New York 12222

The morphology of the margins of Palaeozoic continental crust around the Gulf of Mexico and Caribbean Sea is derived from the distribution of Jurassic evaporites in the Gulf and the distribution of post-Palaeozoic arcs around the Caribbean. The Permian–Triassic position of the continental masses in this region is determined by fitting these margins together to achieve the best agreement of margin morphology and geological continuity. This model is supported by geological observations from the margins and floors of the Gulf and Caribbean and by geometrical considerations derived from continental reconstructions around the Atlantic.

THE plate tectonic evolution of the Gulf of Mexico and Caribbean Sea is poorly understood because a coherent seafloor spreading history based on magnetic anomalies cannot be determined in these basins. Consequently, evolutionary models in this area must be based largely on ambiguous and less precise geological observations from the margins and interpretation of seismic reflection data from the basins. Due to the geological complexities produced by plate kinematics of even geometrically simple systems[1], this approach may not lead to a definitive model, but it constrains the models possible.

Determination of the Permian–Triassic positions of the continents around the Gulf of Mexico and Caribbean Sea is the most critical aspect of all evolutionary models of this region. I propose here a Permian–Triassic reconstruction of the continents around the Gulf of Mexico and Caribbean Sea that emphasises the geometrical constraints imposed by the pre-Mesozoic margin morphology determined for these continents and the fit of the continents around the Atlantic Ocean. The distribution of Jurassic evaporites has a dominant role in determining pre-Mesozoic margins by delimiting the boundaries of Jurassic basins that formed as a result of continental breakup.

Previous models

Although it has been suggested that the Gulf of Mexico existed during the Palaeozoic[2–4], consideration of the stratigraphically controlled seismic reflection data in the Gulf and the geology of the margins of the Gulf and Caribbean support the view that these basins were formed by continental breakup during the Triassic and early Jurassic[6–10]. Several reconstructions have been proposed to close the Gulf of Mexico and Caribbean: the solutions are generally of two types (1) closure by juxtaposing North and South America and relocating Mexico and Central America west of their present position relative to North America[9,11–13] and (2) closure by segmenting and positioning portions of Mexico and Central America between North and South America[6,7,14].

The reconstructions of the first group were supported by the recognition of large strike slip faults in Mexico that provided a means to segment and relocate Mexico and Central America during the late Palaeozoic[4,15,16]. There are also palaeontological, geological and palaeomagnetic data to suggest that South America was very close to North America during the late Palaeozoic[9,11,17]. However, these data lack the resolution to determine precisely the relative positions of North and South America during the late Palaeozoic, and there are several lines of evidence to suggest that they were not contiguous.

One important argument against juxtaposing South America and North America is the absence of a Palaeozoic magmatic arc on either margin. An arc system culminating in late Carboniferous times should have developed as a result of the convergence that formed the Ouachita Mountains. This problem is usually circumvented by placing an inferred arc beneath the thick Mesozoic and Cenozoic sediments of the Gulf Coast (ref. 11, Fig. 2*b*, *c*; ref. 18). However, Wickham *et al.*[19] have shown that this inference is untenable because there are no arc-related, pre-Carboniferous, igneous, metamorphic or sedimentary rocks within the foldbelt; and they interpret the southern margin of North America as a south-facing Atlantic-type margin that

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Present address: Cities Service Company, Energy Resources Group, Cities Service Building, 5100 Southwest Freeway, Box 27570, Houston, Texas 77027.

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collided with a southern continental mass containing the magmatic arc in Pennsylvanian times. Northern South America is unlikely to be this mass because there are no late-Palaeozoic plutonic rocks east of the Venezuelan Andes[20]. The pre-Mesozoic basement to the Coast Ranges of Venezuela seems to be Precambrian rocks of the Guyana Shield that were intruded and deformed during a Cretaceous arc–continent collision[21].

The paucity of Jurassic evaporites along the northern margin of South America also suggests that closure of the Gulf was not achieved by juxtaposing North and South America as suggested by proponents of the first group. Generally, evaporites formed during initial ocean opening lie on both sides of the resultant ocean[10,22]. There are no evaporites on the northern margin of South America that are of comparable extent to the Louann and correlative evaporites of the Gulf of Mexico[10], and this strongly suggests that North and South America were not opposing margins to the same developing ocean basin.

The most convincing argument against juxtaposing South and North America during the latest Palaeozoic is that this requires disruption of the early Triassic fit of the continents around the Atlantic. Because continents are torsionally rigid, the reconstructed positions of the peri-Atlantic continents provide the most powerful constraint on the relative position of North and South America[23]. Positioning South America against the northern margin of the Gulf of Mexico requires unrealistic offsets of corresponding salt basins and fracture zones along the margins of North America and Africa[8,22].

These arguments against the juxtaposition of North and South America support the models of the second group that close the Gulf and Caribbean without this restriction. However, since the models of Carey[14] and Dietz and Holden[6] were proposed, the Greater Antilles (with the possible exception of part of western Cuba) were found to be of post-Palaeozoic age and therefore they need not be considered in a Triassic reconstruction of this area. Freeland and Dietz[7] considered this in their model, but, by removing these post-Palaeozoic continental masses, large gaps were left between North and South America. Subsequent examination indicated that Blake–Bahama Platform, which was included in the reconstruction of Freeland and Dietz[7], is likely to be an early Mesozoic volcanic construction[8,24] and removal of this platform enhances the problem of large unexplained gaps. In addition, the poor morphological and geological fit of the reconstructed Palaeozoic continental masses as proposed by Freeland and Dietz[7] is not convincing.

Collectively these arguments suggest that the Atlantic fits proposed by LePichon and Fox[8] and Sclater *et al.*[25] best satisfy constraints imposed by palaeontological, palaeomagnetic, morphological and petrological data. Tighter fits such as those of VanderVoo *et al.*[9] and Pilger[13] seem unsatisfactory when the geological evolution of opposing margins of North and South America are considered and the more open fit of Bullard *et al.*[26] creates a gap between North and South America that is too large to be filled with the available pre-Mesozoic continent fragments around the Gulf of Mexico and the Caribbean. Additional difficulty in reconstructing this area results from the extensive alteration of the margin morphology of North, Central and South America during the Mesozoic and Cenozoic. Examination of proposed models therefore suggests that a more precise approximation of the Permian–Triassic positions of continents around the Gulf and Caribbean can be obtained by first removing obvious post-Palaeozoic island arcs and sedimentary constructions and then fitting the resultant margins of continental crust back together in the space dictated by the LePichon and Fox[8] fit of the continents around the Atlantic.

Determination of Palaeozoic margins and proposed reconstruction

The distribution of thick Jurassic evaporites is extremely useful in attempting to define the boundaries of pre-Mesozoic crust that resulted from Triassic rifting in the Gulf of Mexico area. The landward boundaries of these deposits are believed to be the walls of rift valley complexes that developed as the continents separated[22]. In the Gulf of Mexico these thick salt deposits seem to lie on oceanic or greatly attenuated continental crust[27–29]. To a first approximation, they can be used to mark boundaries between the Palaeozoic crust of continental thickness and the developing Jurassic ocean basin (Fig. 1). This simplifying assumption requires the removal of the coastal plains and shelves of Texas, Louisiana and Mississippi and places the pre-Mesozoic crustal margin of North America 200–300 km inland of the present shorelines of Texas and Louisiana (ref. 28, Fig. 3). The hinging of younger deposits over this margin is expressed as a fault zone that stretches from Mobile, Alabama, to San Antonio, Texas[29].

The remaining pre-Mesozoic crustal margins around the Gulf can also be delineated by the distribution of evaporites. The western margin of the Yucatan Block in the Isthmus of Techu-

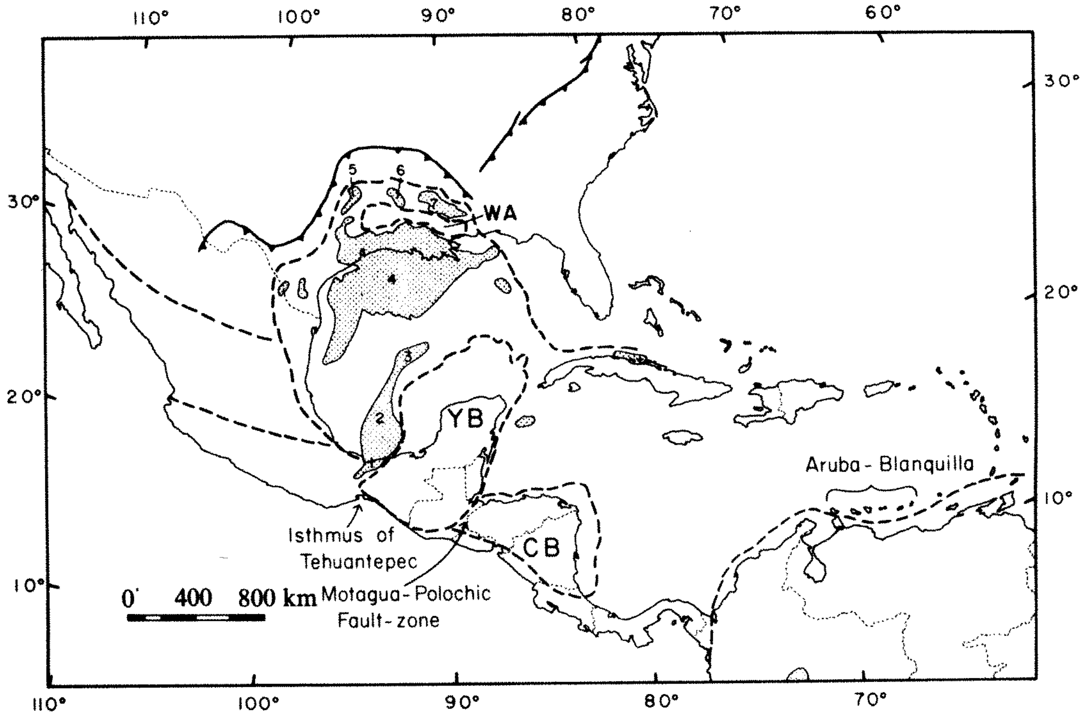

Fig. 1 Map of the Gulf of Mexico and Caribbean Sea showing limits of pre-Mesozoic continental crust (dashed line). YB, Yucatan Block; CB, Chortis Block; WA, Wiggins Arch; 1, Isthmian Basin; 2, Campeche Knolls; 3, Sigsbee Knolls; 4, Gulf Coast Salt Dome Province; 5, East Texas Salt Basin; 6, North Louisiana Salt Basin; 7, Mississippi Salt Basin. Stippling indicates areas of diapiric salt.

antepec is approximately coincident with the eastern edge of the Isthmian Salt Basin, Campeche Knolls and the western limit of Palaeozoic granitic rocks of the Chiapas Mountains near the western border of Chiapas, Mexico (ref. 29, Fig. 1). Seismic reflection studies across the Campeche and Florida Escarpments clearly show pinching out of the Jurassic–Cretaceous (?) Challenger unit (containing the evaporites) against older rocks that form the basement to these shelves[5,30]. These studies and the absence of diapiric salt landward of the Campeche and Florida Escarpments indicate the continental shelves of Yucatan and Florida are underlain by pre-Mesozoic continental crust. Therefore, the Campeche and Florida Escarpments are taken to approximate the Palaeozoic continental crustal margin north and west of Yucatan and Florida respectively (Fig. 1). As shown in Fig. 1, Mexico was segmented into blocks as proposed by Schmidt and Anderson[15].

Determination of the limits of pre-Mesozoic continental crust around the Caribbean is not as straightforward as in the Gulf of Mexico because of the paucity of ocean-opening salts and extensive post-Palaeozoic arc tectonics at the edges of the Caribbean plate. However, most of the circum-Caribbean continental masses are post-Palaeozoic in age, and therefore, only the margins of South America and the Chortis block (Honduras, portions of Guatemala and Nicaragua) need to be considered. The northern margin of South America was formed by rifting in the late Triassic and early Jurassic and experienced intense convergent and strike-slip related deformation after this time[10]. Nonetheless, analysis of the evolution of this margin in Venezuela by Maresch[24] justifies approximating the limits of pre-Mesozoic continental crust between the mainland and the Aruba–Blanquilla Island Chain (Fig. 1).

Two main post-Palaeozoic constructions alter the margins of the Chortis block and are removed in this reconstruction. These are the Nicaraguan Rise, which is a late Mesozoic island arc[31] and the Cenozoic Middle America Arc along the Pacific coast of Guatemala, El Salvador and Nicaragua (Fig. 1). The Motagua–Polochic fault zone marks both the northern boundary of the Chortis block and the southern boundary of the Yucatan block. The eastern and southern limits of the Chortis block are unknown because they are buried under shelf sediments and Tertiary volcanics, but for this reconstruction the eastern limit is approximated by the 1,000-m isobath and the southern limit is approximated by projecting the trend of this isobath across Central America (approximately coincident with the southern Nicaraguan border).

The peri-Gulf and Caribbean blocks, now refined to their pre-Mesozoic shapes, can be assembled as shown in Fig. 2. In making this late Permian–early Triassic assembly opposing margins were matched when possible by closing correlative salt basins. Without this constraint, pre-Mesozoic margins were placed to provide the best morphological and geological fit. Application of this principle to the Gulf of Mexico indicates the extensive salt deposits west and north of Yucatan (Isthmian Basin, Campeche Knolls and the Sigsbee Knolls) correspond in age and extent to those of the northwestern margin (Gulf Coast Salt Dome Province). It is believed these were opposing margins of the developing Jurassic ocean[22,23]. Good morphological agreement of pre-Mesozoic continental boundaries determined from salt distribution not only fixes the relative position of the Yucatan block to the southern margin of North America (Fig. 2), but also justifies the method of margin approximation described earlier. The Wiggins Arch, believed to be a pre-Mesozoic continental horst, is placed north-west of its present position against the North American Palaeozoic margin by closing the Mississippi, North Louisiana and East Texas Salt Basins. The wedge-shaped gap that remains between Yucatan and Florida can most easily be filled by placing the southeastern margin of the Chortis block against the Florida Escarpment.

Positioning the Yucatan and Chortis blocks in this manner entirely fills the gap between North and South America that is produced by the Atlantic reconstructions of LePichon and Fox[8] and Sclater *et al.*[25]. The small elongate gaps south of Florida and Yucatan (Fig. 2) may represent continental crust obliterated during the Bahama–Cuba and Motagua Collisions respectively. The remaining portions of Mexico must be displaced northwestwards of their present position before the opening of the Gulf. Whether or not this was accomplished by displacement along the Mohave–Sonora shear zone and the site of the Mexican volcanic zone (ref. 15, Fig. 1) or some other boundary must await further study. Palaeomagnetic data from Mexico and Central America[33] are difficult to interpret because of extensive post-Jurassic deformation in these regions. Pre-Mesozoic portions of Cuba are assumed here to be small and, therefore, do not alter the positions of the major blocks, but the present position of Cuba relative to the Yucatan and Chortis blocks suggests placing this continental crust at the juction of these blocks with the North American margin (area C in Fig. 2).

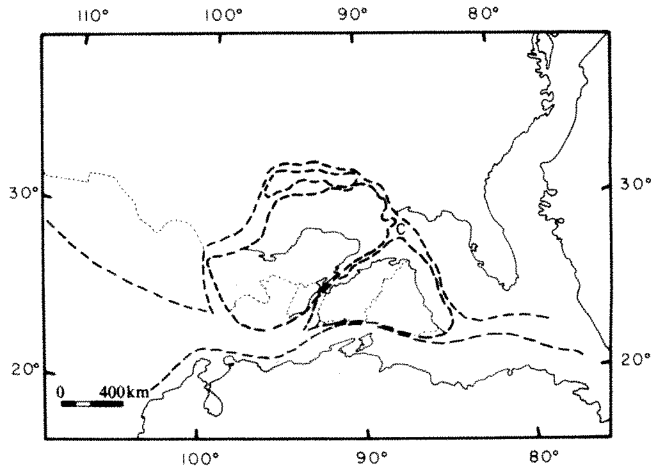

Fig. 2 Reconstructed positions of the continents around the Gulf of Mexico and Caribbean Sea during the early Triassic. C, Location of pre-Mesozoic portions of Cuba.

Discussion and implications

This reconstruction satisfactorily explains many features observed around the Gulf and Caribbean region. Most notably it explains the late Triassic to early Jurassic rifting event seen on the margins of the Gulf of Mexico and northern South America without juxtaposing North and South America and without leaving large gaps between these two continents. The Triassic La Quinta and correlative redbeds of Venezuela and Colombia and the early Jurassic Apoteri tholeiitic basalt flows of Guyana and Brazil[34] suggest that the Triassic graben complex associated with the Gulf–Atlantic opening extended into South America; but the subsequent seafloor spreading that occurred in some of these graben to open the oceanic basins between North and South America was not synchronous. Continuity of the Challenger seismic unit across the central Gulf[5,29] indicates that seafloor spreading in the Gulf ceased at least by the end of Challenger deposition. Therefore, the Gulf was largely created in Lias–Dogger times. However, subsidence of the northern margin of South America which began in the late Jurassic[21] suggests seafloor spreading in the Caribbean began in Malm times and continued into the Cretaceous.

The presence of extensive ocean-opening evaporites in the Gulf of Mexico and the paucity of evaporites in the Caribbean area may be explained by this geometry and timing of basin development. Figure 2 suggests that Mexico and Florida served as structural barriers against open ocean circulation in the Gulf as the Yucatan and Chortis blocks moved away from North America until Medial Jurassic times. At this time, communication with the developing Atlantic Ocean was established through the old Bahama channel and extensive evaporite deposition ceased in the Gulf. Apparently no such barriers restricted circulation in the developing Caribbean which was in constant communication with the Pacific and Atlantic Oceans as it opened.

This reconstruction also suggests that the Palaeozoic magmatic arc resulting from closure of the Ouachita Ocean to form the Ouachita Mountains is likely to be found as the basement to Mesozoic sediments in Yucatan. Discovery of rhyolite of Silurian age showing a Mississippian metamorphic event (collision?) in the Yucatan No. 1 well of the Central Yucatan Peninsula[35] supports this view. I suggest that the granites and volcanic rocks that underlie Lower Jurassic (?Triassic) redbeds of Yucatan described by Lopez-Ramos[36] may also belong to this arc system. This arc was probably the source of middle Palaeozoic granitic and rhyolitic clasts and andesite tuffs in the Upper Palaeozoic flysch sequences of the Ouachitas[37].

Further support for this configuration is found in the Palaeozoic rocks of northeastern Mexico. The pre-Mesozoic schist, metaquartzite, granite and Devonian gneiss of eastern Coahuila and Nuevo Leon, cannot be correlated with Palaeozoic terrains in Chihuahua, Mexico, or the Ouachita belt in the southern US[37], but these rocks are remarkably similar to the pre-Mesozoic schist, granite and gneiss terrains of the Chiapas Mountains of the Isthmus of Tehuantepec[25]. I suggest that these terrains were contiguous during the Palaeozoic (Fig. 2).

The pre-Mesozoic rocks (predominately phyllite, marble and schist)[35] of the Chortis block are not easily correlated with others in the Gulf or Caribbean region and its position in this reconstruction is largely based on morphology. However, phyllite and marble dredged from the Yucatan Channel near the north-east corner of the Yucatan Peninsula[38] and schist found in the Basil Jones No. 1 well in Belice[36] are possible correlatives and as such they support location of the Chortis block as proposed in Fig. 2.

Correlation of pre-Mesozoic terrains between northern South America, the Yucatan block and the Chortis block is difficult because of the complex history of the northern South American margin. In general, Upper Palaeozoic rocks are restricted to a smooth curvilinear belt that extends from the Appalachians into the Andes, but these correlations are too crude to fix precisely the position of South America. Nevertheless, continuity of Palaeozoic terrains, good morphological agreement between South America and the other continental blocks (Fig. 2), and the Mesozoic tectonic history of northern South America[10,21] support this reconstruction.

The margins of the Yucatan, Chortis and Mexican blocks probably originated as intracontinental transform fault zones that were coaeval with the collision of Laurasia and Gondwanaland. Continued crustal shortening after collision probably caused shuffling of these blocks resulting in the complex late Palaeozoic to early Mesozoic deformation in Mexico and Central America as suggested by Dengo[35]. I thank K. C. Burke, S. E. DeLong, J. F. Dewey, P. J. Fox, W. S. F. Kidd, R. E. Livaccari, A. M. C. Sengör and J. B. Stroup for useful suggestions, R. Carosella for assistance in preparing this paper, and Kevin Burke and Bill Kidd for helpful discussions. This work was supported by NSF grants EAR 7514254 and EAR 7803319 awarded to Kevin Burke.

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Abelson murine leukaemia virus protein is phosphorylated *in vitro* to form phosphotyrosine

Owen N. Witte, Asim Dasgupta & David Baltimore

Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

The Abelson murine leukaemia virus protein (P120) can become phosphorylated in vitro *by [γ-^{32}P]ATP. The protein has been purified from cell membranes to the point that in specific conditions virtually all of the incorporated ^{32}P is in P120. The reaction is stimulated by Mn^{2+} and Mg^{2+} but not Ca^{2+} and is very rapid even at 0 °C. The phosphate is linked to P120 at tyrosine, a linkage not previously reported for a phosphorylation reaction. Phosphorylation may be involved in the transforming activity of viruses that cause leukaemia as well as sarcomas.*

ABELSON murine leukaemia virus (A-MuLV) is a defective retrovirus that causes a thymus-independent leukaemia[1,2]. The leukaemic transformation can be carried out in cultures of bone marrow, fetal liver or spleen cells and many of the resultant cell lines have the characteristics of pre-B-lymphocytes[3]. Continuous lines of fibroblastic cells can also be transformed by the virus[4].

The A-MuLV genome is a hybrid consisting of about 25% of the genome of Moloney murine leukaemia virus (M-MuLV) flanking an insert of 3.6 kilobases of sequence that is homolo-

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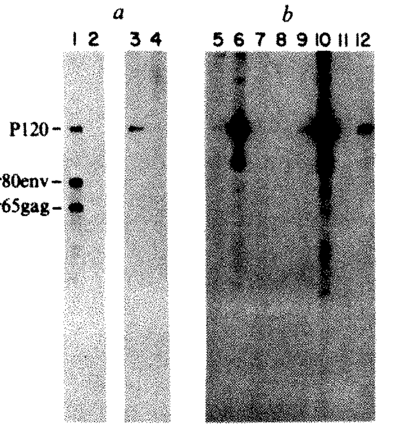

Fig. 1 Phosphorylation of P120 in an immunoprecipitate. *a*, 2M3/M cells (an A-MuLV-transformed BALB/c lymphoid cell line superinfected with Moloney-MuLV[7]) were labelled with ^{35}S-methionine (2.5×10^6 cells, 100 μCi ^{35}S-methionine in 3 ml Dulbecco's minimal essential medium minus methionine for 1 h). Cells were extracted and clarified for immunoprecipitation[7,9], then one half was reacted with 5 μl anti-A-MuLV tumour serum[9] (lane 1) and one half with 5 μl normal mouse serum (lane 2). Precipitates were collected with *S. aureus*, denatured and analysed on a SDS 10% polyacrylamide gel developed by fluorography[25,26]. An equal number of unlabelled 2M3/M cells was extracted and clarified as above and immunoprecipitated with anti-A-MuLV tumour serum (lane 3) or normal mouse serum (lane 4). The precipitates were washed with 20 mM Tris-HCl, *p*H 8.0, by centrifugation, and resuspended in 50 μl of 20 mM Tris, *p*H 8.0, 10 mM $MgCl_2$, containing 1 μCi [γ-^{32}P]ATP (NEN, 2,300 Ci mmol^{-1}) for 10 min at 30 °C, washed, denatured and analysed by electrophoresis as described above. *b*, For each lane, 1×10^6 2M3 cells (an A-MuLV transformed lymphoid non-producer cell line) were extracted and clarified, then immunoprecipitated with 5 μl of a polyvalent goat anti-M-MuLV virion serum[9]. Individual precipitates were tested for phosphorylation of P120 as described above, with the following cations present: lane 5, 1 mM $CoCl_2$; lane 6, 10 mM $CoCl_2$; lane 7, 1 mM $CaCl_2$; lane 8, 10 mM $CaCl_2$; lane 9, 1 mM $MnCl_2$; lane 10, 10 mM $MnCl_2$; lane 11, 1 mM $MgCl_2$; lane 12, 10 mM $MgCl_2$.

gous to the DNA of normal mouse cells[5,6]. Only a single protein of the A-MuLV genome has been identifiable using a wide range of sera; in the prototype A-MuLV strain, it is called P120 and represents a fusion of the N-terminal segment of molecular weight (MW) 30,000 of the M-MuLV gag protein precursor to protein of MW 90,000 that is non-viral[7,8]. An antiserum specific for the non-MuLV-related portion of P120 has been prepared and reacts with protein of MW 150,000 in normal mouse cells[9,10]. Thus, the non-viral portion of the P120 is closely related to a normal cell protein and is encoded by information that has at least partial homology to normal cell DNA.

Because no conditional mutants of A-MuLV are known, it has not been possible to prove that the P120 is the viral transforming protein, but because it is the only defined product of the genome and because of its association with A-MuLV-induced transformed cells, it has been assumed to be responsible for transformation by A-MuLV[2,3]. The antiserum that recognises the non-M-MuLV-related portion of the P120 also reacts with the external surface of the plasma membrane of A-MuLV-transformed cells; thus, the P120 seems to have a portion exposed outside the cell[9]. Anti-M-MuLV sera do not react with the surface of A-MuLV-transformed, non-producer cells, indicating that the M-MuLV gag determinants are inside the cell. The protein therefore seems to be a transmembrane protein of the plasma membrane. P120 is not detectably glycosylated and is not cleaved by protease treatment of live cells; most of the polypeptide is therefore probably not exposed to the outside of the cell.

P120 is phosphorylated[7]. This observation led us to examine whether its properties might be related to those of the Rous sarcoma virus transforming protein, pp60 src. That protein, which is recovered as a phosphoprotein from infected cells, has been shown to catalyse the incorporation of ^{32}P from [γ-^{32}P]ATP into the heavy chain of immunoglobulins that bind to it [11,12]. The original demonstration of phosphorylation by pp60src was carried out with immunoprecipitates bound to *Staphylococcus aureus* and we therefore began our studies with such a system.

P120 phosphorylation in immunoprecipitates

To examine P120 for phosphorylation activity, an immunoprecipitate was incubated with [γ-^{32}P]ATP essentially as described by Collett and Erikson[11] for the Rous sarcoma virus transforming protein. Lysates were prepared from ^{35}S-methionine-labelled or from unlabelled A-MuLV-transformed lymphoid cells (2M3/M) (the cells also contained M-MuLV proteins because they were superinfected with M-MuLV). The extracts were mixed with control serum or antiserum directed

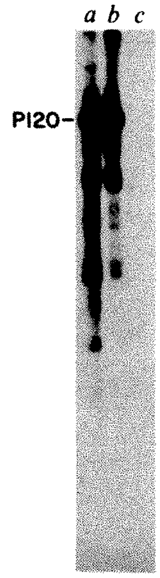

Fig. 2 Preparation and specificity of a soluble system that incorporates phosphate into P120. A frozen pellet of 2×10^9 2M3 cells was extracted at 0–4 °C with a detergent extraction mix of 100 ml of 10 mM NaH_2PO_4–Na_2HPO_4, *p*H 7.5, 100 mM NaCl, 1.0% Triton X-100, 0.5% Na deoxycholate, 0.1% SDS, 10% glycerol, 1 mM EDTA and 5 mM phenylmethylsulphonyl fluoride. This was clarified at 20,000 r.p.m. for 2 h in a Sorvall RC-5 refrigerated centrifuge. To the supernatant fluid was added 20 ml packed volume of DEAE-cellulose in the same buffer. The supernatant was recovered by centrifugation and then batch absorbed to 20 ml packed volume of phosphocellulose. P120 binds to phosphocellulose in these conditions as monitored by immunoprecipitation of ^{3}H-leucine-labelled cellular lysates (data not shown). The resin was washed with the extraction buffer and P120 eluted with 30 ml of 0.5 M NaCl in extraction mix. The eluted material was dialysed against 10 mM KH_2PO_4–K_2HPO_4, *p*H 8.0, 100 mM NaCl and 25% glycerol. The dialysate was reconcentrated by absorption to phosphocellulose on a small column (1.2 × 10 cm) and elution into 4 ml of 0.5 M NaCl extraction buffer minus the SDS, containing 25% glycerol. This preparation represented 3% of the total protein in the original lysate and the recovery of P120 (estimated from experiments using labelled cellular lysates) was about 80%. This preparation was stable for over 6 weeks when stored at −20°C. The enzyme preparation (5 μl) was assayed in a 50-μl reaction mix containing 20 mM Tris HCl, *p*H 8.0, 10 mM $MnCl_2$ and 1 μCi [γ-^{32}P]ATP for 10 min at 30 °C. One portion was analysed by electrophoresis to display all the phosphorylated proteins (lane *a*). An equal portion was immunoprecipitated with 5 μl of goat antiserum to total M-MuLV virion proteins (lane *b*; as in Fig. 1 and refs). A third equal portion was treated with 5 μl of normal goat serum (lane *c*). Analysis on SDS 10% polyacrylamide gel and autoradiography as in Fig. 1.

towards the A-MuLV-specific region of the P120 protein as well as the helper virus proteins. Immunoprecipitates were collected on fixed *S. aureus* immunoabsorbant. The precipitates from unlabelled cells were incubated with [γ-^{32}P]ATP and Mg^{2+} for 10 min at 30 °C and washed. The ^{35}S-labelled samples and ^{32}P-labelled samples were then denatured and analysed by SDS-polyacrylamide gel electrophoresis. The ^{35}S-labelled sample had, as expected from previous work[7,9], a strong band of P120 precipitated by immune but not normal serum (Fig. 1*a*, lanes 1, 2). The ^{32}P-labelled precipitates also showed a band of radioactivity at MW 120,000 when precipitated by the immune but not the normal serum (Fig. 1*a*, lanes 3, 4). This experiment has been carried out with a variety of antisera directed at the gag or A-MuLV-specific regions of the P120 (refs 7, 9), and with a variety of A-MuLV-transformed cells that lacked the M-MuLV proteins, and labelling of the 120,000-MW protein has always occurred. Thus, the ^{32}P-labelled protein has the immunological as well as the electrophoretic characteristics of P120 and therefore seems to be P120 itself. No labelling of immunoglobulins was evident nor was there labelling of the M-MuLV proteins Pr65gag and Pr80env that were present in the immunoprecipitate (Fig. 1*a*, lane 3).

Table 1 Kinetic stability of the phosphate covalently bound to P120

Cation	First incubation [ATP]	First incubation c.p.m. × 10^{-3}	Second incubation [ATP]	Second incubation c.p.m. × 10^{-3}
Mg	10 nM	21.2	10 nM	17.9
	10 nM	21.2	500 µM	15.6
	500 µM	1.6	ND	
Mn	10 nM	63.1	10 nM	59.7
	10 nM	63.1	500 µM	56.4
	500 µM	2.8	ND	

All reactions were carried out at 30 °C for 10 min in 20 mM Tris, *p*H 8.0, containing Mg or Mn at 10 mM in 50 µl final volume using 10 µl of phosphocellulose-purified enzyme. The first incubation contained either only radioactive [γ-^{32}P]ATP (NEN, 2,400 Ci mmol^{-1}) at 10 nM final concentration or the same amount of radioactive ATP plus added unlabelled ATP to 500 µM final concentration. The total c.p.m. incorporated was determined by cold 5% trichloroacetic acid (TCA) precipitation. For the second incubation, duplicate reactions from the first set of incubations using the 10 nM ATP condition were reincubated (10 min, 30 °C) with no additional ATP or with unlabelled ATP to 500 µM final concentration and c.p.m. incorporated determined as above. ND, Not determined.

To examine the cation specificity of ^{32}P incorporation, different ions were tested at 1 and 10 mM. Mn^{2+} and Co^{2+} were most effective, Mg^{2+} was less effective and Ca^{2+} supported no detectable incorporation (Fig. 1*b*). In all cases where incorporation was seen, 10 mM cation was preferable to 1 mM. Ca^{2+} added to Mn^{2+} did not inhibit incorporation (data not shown). These effects were similar over an ATP concentration range of 20 nM–1 mM.

A variety of other variables were examined and their effects monitored semi-quantitatively by estimating band intensities following electrophoresis. [γ-^{32}P]GTP was found to be a ^{32}P donor to P120 but at much lower efficiency than [γ-^{32}P]ATP. Labelling with [γ-^{32}P]ATP (at 10–20 nM) was complete within about 1 min even at 0 °C and the yield was about the same at 37 °C or 0 °C. Variation of the *p*H from 6 to 9 had little effect. No effect was noted when cyclic AMP, cyclic GMP or any of the four ribonucleoside monophosphates or diphosphates was added at 10–500 µM. When 10–50 µM of any of the four ribonucleoside triphosphates was added to 10 nM [γ-^{32}P]ATP in the presence of Mg^{2+}, a threefold stimulation of incorporation of ^{32}P into P120 was seen. This effect is most remarkable for ATP because ATP will directly dilute the specific activity of the [γ-^{32}P]ATP. The result may imply a regulatory role for nucleoside triphosphates (NTPs) in the reaction. Less stimulation by NTPs occurred in the presence of Mn^{2+}.

A soluble system for P120 phosphorylation

Because the behaviour of P120 in immunoprecipitates might be misleading, we have developed a partial purification scheme for P120 starting with a detergent extract of infected cells. The purification was monitored using ^{3}H-leucine-labelled extracts and immunoprecipitation with specific antiserum as in Fig. 1, lane 1. P120 did not bind to DEAE-cellulose but bound to phosphocellulose and could be eluted at about 0.5 M NaCl. In the crude extract as well as in the phosphocellulose-purified material, ^{32}P incorporation from [γ-^{32}P]ATP into P120 could be demonstrated by immunoprecipitation after the reaction. After phosphocellulose purification, as much as 85% of the acid-insoluble ^{32}P could be immunoprecipitated. At that stage, the electrophoretic profiles of equal amounts of the total reaction product (Fig. 2, lane *a*) and the immunoprecipitated product (Fig. 2, lane *b*) were quite similar. No radioactive bands were evident when normal serum was used in place of the immune serum (Fig. 2, lane *c*), although about 5% of the incorporated ^{32}P appeared in the precipitate after treatment with normal serum. The various labelled bands other than P120 that were precipitated by immune serum probably represented labelled degradation products of P120 that were produced during the incubation. This degree of specific phosphorylation by the phosphocellulose fraction has been confirmed in three separate preparations and a wide variety of reaction conditions.

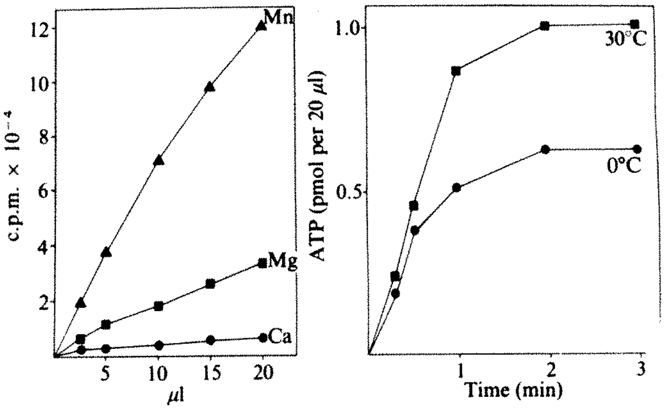

Fig. 3 Properties of the soluble P120 phosphorylation reaction. *a*, Varying amounts of an enzyme preparation prepared as described in Fig. 2 legend were reacted for 10 min at 30 °C in a final 50 µl volume containing 1 µCi [γ-^{32}P]ATP, 20 mM Tris, *p*H 8.0, and 10 mM CaCl$_2$ (○), MgCl$_2$ (□) or MnCl$_2$ (△). Labelled proteins were precipitated with 1 ml of cold 5% TCA, collected and washed by Millipore filtration and counted in 5 ml of Bray's solution. *b*, An enzyme preparation (10 µl) was reacted in 50 µl final volume containing 50 µm ATP, 10 mM MnCl$_2$ and 20 mM Tris, *p*H 8.0, at 0 °C or 30 °C for the times shown. Reactions were stopped by addition of cold 5% TCA, collected and counted as above. Longer times of reaction (5–30 min) showed no increase above the 2-min level (data not shown).

Although this fraction is enriched for P120, analysis of its total protein content by Coomassie blue staining of SDS-polyacrylamide gels showed more than 50 separate bands of varying intensity (data not shown). P120 was so minor a component that we could not identify a specific band of P120 protein in this mixture.

Characterisation of the soluble phosphorylation reaction

Because the majority of ^{32}P incorporated by the phosphocellulose fraction was incorporated into specifically immunoprecipitable material, the properties of this fraction were investigated further using an acid precipitation assay. The yield of the incorporation was a linear function of the concentration of added protein but, as in the immunoprecipitate reaction, Mn^{2+} was greatly preferred over Mg^{2+} and Ca^{2+} was virtually inactive (Fig. 3*a*). The reaction yield was a function of the ATP concentration and was half maximal at about 10 μM. At 50 μM ATP, the reaction showed a rapid time course at either 0 °C or 30 °C but at 30 °C 30–40% more incorporation was evident than at 0 °C (Fig. 3*b*). Cyclic AMP or cyclic GMP did not affect the reaction (data not shown).

Because sufficient P120 could be added to incorporate ^{32}P corresponding to about 10–15% of added [γ-^{32}P]ATP into acid-precipitable material, the fate of the substrate was examined. Preliminary experiments showed that at 30 °C ATPase and phosphatase activities in the phosphocellulose eluate, possibly unrelated to P120, degraded much of the added ATP. At 0 °C and with Ca^{2+} added, these were suppressed without affecting P120 labelling and therefore these conditions were used for the experiments. Equal amounts of enzyme were incubated at 0 °C for 15 min with equal molar amounts of either [γ-^{32}P]ATP (Fig. 4, lanes *a–c*) or [α-^{32}P]ATP (Fig. 4, lanes *d–f*) using Ca^{2+} (lanes *a*, *d*), Mn^{2+} (lanes *b*, *e*) or Mn^{2+} and Ca^{2+} (lanes *c*, *f*); portions of the reactions were analysed by ascending chromatography. With Ca^{2+} alone, some degradation of both [γ-^{32}P]ATP to P_i and [α-^{32}P]ATP to ADP/AMP was evident but with Mn^{2+} present much more ADP was found than in its absence. Also, when Mn^{2+} was added, appreciable radioactivity from [γ-^{32}P]ATP became protein bound and remained at the origin. Approximately the same fractions of the ^{32}P in [γ-^{32}P]ATP became protein bound as the fraction of [α-^{32}P]ATP that was converted to ^{32}P-ADP. Thus, only the γ-phosphate of ATP seemed to participate in the labelling of P120, and ADP was released.

To examine whether the ^{32}P incorporated into P120 was turning over during the reaction, the [γ-^{32}P]ATP (10 nM) was diluted with a great excess of unlabelled ATP (500 μM) after 10 min of reaction and another 10 min of incubation was carried out (Table 1). With either Mg^{2+} or Mn^{2+} present, little loss of radioactivity was evident during the second incubation, although the excess of added ATP was sufficient to reduce the initial incorporation of ^{32}P by 10- to 30-fold. Thus, the phosphate incorporated in P120 was not subsequently released as acid-soluble products.

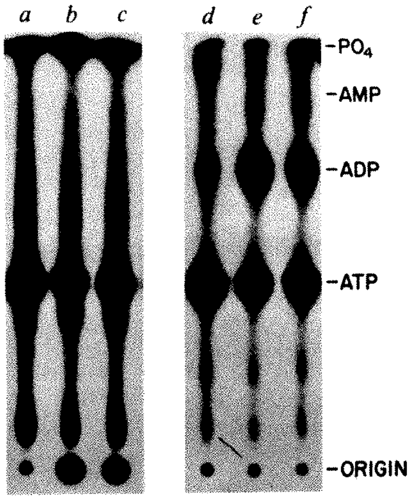

Fig. 4 Fate of ATP during the P120 phosphorylation reaction. Phosphorylation reactions (100 μl final volume) contained 20 μl of enzyme preparation and either 1 μCi of [γ-^{32}P]ATP (lanes *a–c*) or [α-^{32}P]ATP (lanes *d–f*). Both ATPs were adjusted to 600 Ci $mmol^{-1}$. Also present were 20 mM Tris, *p*H 8.0, and 10 mM $CaCl_2$ (lanes *a*, *d*), 10 mM $MnCl_2$ (lanes *b*, *e*) or 10 mM $CaCl_2$ plus 10 mM $MnCl_2$ (lanes *c*, *f*). Reactions were at 0 °C for 15 min and were then diluted 20-fold into excess EDTA. Aliquots of 1 μl of each were analysed on polyethylenimine-coated thin layer plates (Brinkman, PEI 300) by ascending chromatography in 0.85 M K_2HPO_4, adjusted to *p*H 3.5. Positions of ^{32}P-PO_4 and marker nonradioactive AMP, ADP and ATP (visualised under UV light) are shown.

Identification of the P120-phosphate bond as phosphotyrosine

As an initial characterisation of the bond between the protein and the phosphate, the acid and alkali stabilities of the product were studied (Table 2). Neither 0.1 M HCl nor 0.1 M NaOH released any acid-soluble radioactivity after 30 min at 37 °C. Even 1 M HCl at 37 °C released no detectable ^{32}P, but 2 M HCl at 100 °C for 20 h converted all the protein-bound ^{32}P to inorganic phosphate. These properties were inconsistent with the acid lability of phosphate linkages to nitrogen[13] and the general lability of acylphosphate linkages[14]. Furthermore, the 2 M HCl hydrolysis should have released some phosphoserine or phosphothreonine if they were present[11].

All the radioactivity could be released from ^{32}P-P120 as $^{32}PO_4$ by treatment with calf intestinal phosphates or bacterial alkaline phosphatase (Fig. 5). This cleavage could be inhibited by the addition of pure phosphomonoester compounds like phosphoserine (data not shown). In this experiment, the ^{32}P-P120 was predigested with chymotrypsin in an attempt to increase the sensitivity of ^{32}P-P120 to hydrolysis but in other experiments this has proved unnecessary. Thus, the bond between the ^{32}P and the protein behaved like a phosphomonoester.

To search for phosphomonoesters that were more labile to acid than phosphoserine or phosphothreonine, a preparation of ^{32}P-P120 from the *in vitro* phosphorylation system was hydrolysed at 100 °C in 6 M HCl for 0, 2, 4 or 6 h and the products were separated by *p*H 3.5 electrophoresis (Fig. 6, lanes *a–d*). Before hydrolysis, the radioactivity remained at the origin but at all times of hydrolysis extensive production of free $^{32}PO_4$ was evident. Also evident after hydrolysis was a prominent spot co-migrating with a phosphotyrosine standard. The apparent

Table 2 Stability of the phosphate bond in *in vitro* phosphorylated P120

Treatment*	% Protein bound†	% Free PO4‡
H_2O, 37 °C, 30 min	100	ND
0.1 M HCl, 37 °C, 30 min	99	ND
0.1 M NaOH, 37 °C, 30 min	101	ND
1 M HCl, 37 °C, 30 min	102	ND
H_2O, 0 °C, 20 h	ND	<5
2 M HCl, 100 °C, 20 h	ND	>95

* 8,000 c.p.m. of *in vitro* phosphorylated P120-P prepared and washed free of unincorporated [γ-^{32}P]ATP as described in Fig. 6 legend was treated in 200 μl of the solution indicates.

† Samples were precipitated with cold 5% TCA, collected and washed on Millipore filters and counted in Bray's solution. The control is set at 100%.

‡ Following hydrolysis, the samples were concentrated by lyophilisation and analysed by high voltage electrophoresis at *p*H 3.5 as described in Fig. 6 legend. Autoradiograms with orthophosphate markers were used to locate the spot of free phosphate, which was excised and counted by Cerenkov radiation. ND, Not determined.

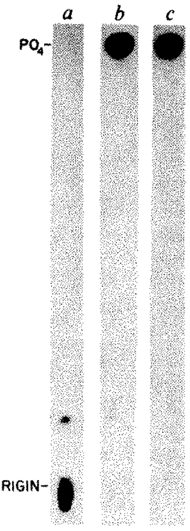

Fig. 5 Susceptibility of ^{32}P-P120 to phosphatase. *In vitro* phosphorylated P120 prepared in a soluble reaction (10 mM $MnCl_2$, 1 μCi [γ-^{32}P]ATP, 20 mM Tris, *p*H 8.0, 20 μl enzyme preparation, 0 °C, 10 min) was precipitated with cold TCA and washed with ethanol to remove unincorporated ATP. The pellet was resuspended in 100 mM NH_4HCO_3, digested with α-chymotrypsin (10 μg ml^{-1}, 37 °C, 12 h), and then boiled for 5 min. Portions containing 2,500 c.p.m. were untreated (*a*), digested with calf intestinal phosphatase (0.01 units, Boehringer Mannheim (*b*) or bacterial alkaline phosphatase (0.01 units, Worthington) (*c*) in 20 mM NH_4HCO_3 and 5 mM $MgCl_2$ for 30 min at 37 °C. Aliquots of 5 μl were analysed by high voltage electrophoresis (Savant) in pyridine acetic acid (*p*H 3.5) on Whatman 3-MM paper at 2,400 V for 1 h. The position of a ^{32}P-PO_4 standard is noted.

phosphotyrosine was eluted and chromatographed with an internal phosphotyrosine marker (Fig. 7). Phosphoserine and phosphothreonine migrated closer to the front than phosphotyrosine in this system. Trace amounts of a compound migrating with phosphoserine were also released by hydrolysis of this preparation (Fig. 6, lanes *b–d*) but recovery of phosphoserine has been variable and always small.

Phosphotyrosine is known to be more acid labile than either phosphoserine or phosphothreonine[15]. Thus, although the yield of ^{32}P-phosphotyrosine was never more than 40–50% of the input radioactivity in ^{32}P-P120, the virtual lack of phosphoserine and phosphothreonine, coupled with the sensitivity of all the ^{32}P to phosphomonoesterases (Fig. 5), is consistent with the interpretation that almost all ^{32}P in ^{32}P-P120 is actually in phosphotyrosine linkage. This report, and a recent independent observation on middle T antigen of polyoma virus[20], appear to represent the first direct demonstrations that phosphotyrosine can occur as a distinct entity in phosphoproteins, although three examples of a tyrosine-*O-P*-nucleotidyl linkage have been reported (refs 16–19 and J. Wang, personal communication). Although the occurrence of phosphotyrosine may represent a unique or very rare situation, the possibility that this linkage is commonly missed because of its acid lability should be considered.

P120 has previously been shown to be labelled in cells exposed to $^{32}PO_4$. To examine whether this *in vivo* phosphorylation occurs at tyrosine residues, *in vivo*-labelled ^{32}P-P120 was hydrolysed in acid using the same conditions as described for the *in vitro*-phosphorylated protein (Fig. 6, lanes *e–h*). The *in vivo* label was recovered mainly as phosphoserine or free phosphate. Virtually no phosphotyrosine was evident and the small spot that migrated just ahead of phosphotyrosine had too little radioactivity to be analysed further. It thus seems that most of the *in vivo* phosphorylation occurs at serine residues, but the extensive release of ^{32}P as inorganic phosphate could suggest that more labile bonds are also present. P120 contains the M-MuLV gag protein pp12 which is known to have serine residues that can be phosphorylated[19]; perhaps some or all the phosphoserine in the *in vivo*-labelled P120 is located in the pp12 region of the molecule.

Interpretation of the P120 phosphorylation reaction

The properties of the P120 phosphorylation reaction described here are unique and not easily interpretable. First, the linkage of phosphate to tyrosine is a unique one and thus offers no clue to its function. We have recently become aware that an apparent autophosphorylation of polyoma virus middle T antigen has also been shown to involve phosphotyrosine. Thus, phosphotyrosine is involved in more than one viral protein thought to play a part in transformation[20]. Second, the reaction seems to be an autophosphorylation. Although it is difficult to be certain whether any other proteins are involved in the reaction, no protein other than P120 is present in the immunoprecipitates at even 10% of the level of P120 and yet rapid phosphorylation of P120 occurs in the immunoprecipitate even at 0 °C. The M-MuLV proteins (evident in Fig. 1, lane 1) are not involved because an immunoprecipitate from cells lacking M-MuLV proteins was active in P120 phosphorylation. Third, the rapid reaction even at 0 °C is suggestive of the formation of an intermediate in an enzyme reaction. The stability of the phosphate-protein linkage after dilution of the [γ-^{32}P]ATP indicates that the reaction as it occurs is not catalytic phosphate transfer. It is, of course, quite possible that the protein kinase reaction is meant to produce a stable tyrosine phosphorylation but it is significant that P120 from cells labelled with ^{32}P-PO_4 had little or no phosphotyrosine.

One attractive possibility is that P120 is a phosphotransferase or phosphatase, but that in the assay conditions used here it is

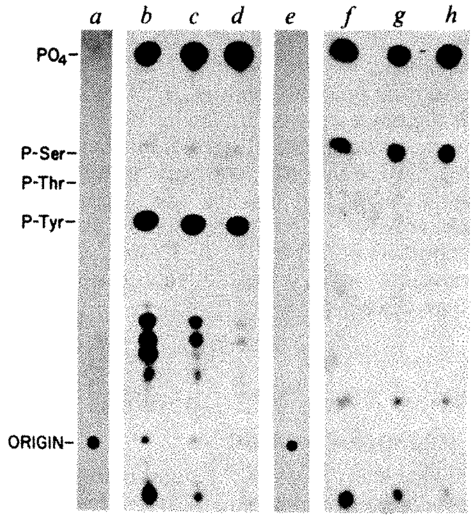

Fig. 6 Analysis of acid hydrolysates of phosphate-labelled P120 made *in vitro* and in cells. About 5,000 c.p.m. of *in vitro* phosphorylated P120 (0 °C, 10 mM $MnCl_2$ reaction conditions as for Fig. 3) recovered by immunoprecipitation, was washed with TCA and ethanol to remove unincorporated [γ-^{32}P]ATP and was either left untreated (*a*) or hydrolysed with 6 M HCl at 100 °C, in a sealed ampoule for 2 h (*b*), 4 h (*c*) or 6 h (*d*). About 2,000 c.p.m. of *in vivo* phosphorylated P120 (2 × 10^6 2M3 cells labelled in phosphate-free medium with 200 μCi of ^{32}P-PO_4 for 3 h, lysed and immunoprecipitated as for Fig. 1, lane 1) was washed with TCA, ethanol and was either left untreated (*e*) or hydrolysed with 6 M HCl at 100 °C for 2 h (*f*), 4 h (*g*) or 6 h (*h*). Samples were lyophilised, resuspended in 5 μl water and a portion analysed by high voltage electrophoresis (*p*H 3.5) as in Fig. 5. The position of marker phosphoserine, phosphothreonine and phosphotyrosine[15] was determined by ninhydrin staining.

unable to transfer the phosphate from its linkage to tyrosine. By this postulate, the P120 phosphorylation could be a partial reaction. Possibly, we need to find the appropriate substrate for phosphate transfer, but two other interpretations should be considered: (1) that removal of P120 from its normal association with membranes has prevented a second step that it ordinarily carries out; thus, we might trap an intermediate state. (2) P120 might initially form a bond other than phosphotyrosine but might transfer the phosphate to tyrosine because of an altered configuration due to its removal from membranes. The P120 is a fused product of viral information and part of a cellular gene; possibly the tyrosine is in the viral portion and is not normally available in the cellular product. We have previously reported that only extracts of A-MuLV-transformed cells could function in *trans* to label heat-inactivated A-MuLV protein of another strain when both were mixed before immunoprecipitation and phosphorylation assay[21]. Extracts of normal cells or cells transformed by other agents were ineffective. One possibility is that the active form for phosphorylation of P120 is a dimer or higher multimer of like subunits. Such preformed complexes would account for the rapid time course and allow for the rescue of heat-inactivated subunits by reforming complexes with native subunits provided in *trans*.

Comparison with Rous sarcoma virus pp60src

Although A-MuLV selectivity transforms lymphoid and occasionally other haematopoietic cells in infected animals, it will transform cultured fibroblastic cell lines[3,4]. Thus, in culture, it can behave like a sarcoma virus and it is not too surprising that the P120 has some parallels with the Rous sarcoma virus protein (pp60src). That protein, like P120 (ref. 9), is membrane associated[22], suggesting that transformation events may often take place at the cell surface. The A-MuLV protein is known to have a portion extending out of the cell because antisera that immunoprecipitate P120 also stain the exterior membrane of live, A-MuLV-transformed cells[9]. With pp60src, the antisera only label the internal surface of the plasma membrane but this could reflect differences in the determinants recognised by the available sera rather than fundamental differences in the organisation of the protein.

Another parallel between pp60src and P120 is that both are involved in phosphorylation reactions. The major activity of pp60src, however, is *trans*-phosphorylation of acceptor proteins (such as immunoglobulin, phosvitin). By contrast, P120 causes little or no labelling of added protein (data not shown) or of the immunoglobulin that binds to it. Whether this difference is fundamental or is a peculiarity of the specific systems is unclear but some phosphorylation of pp60src itself has been noted[23].

Although pp60src has not been reported to form phosphotyrosine, this question needs further investigation. Other properties of the pp60src and P120 reactions have similarities, the major one being that both reactions occur rapidly at 0 °C. Also, Ca^{2+} will support neither reaction but is not inhibitory to either one.

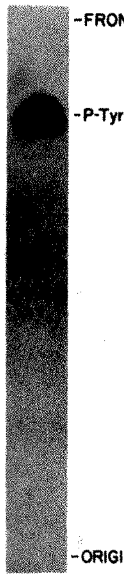

Fig. 7 Co-chromatography of phosphotyrosine with the major phosphorylated amino acid recovered from *in vitro* phosphorylated P120. Material from the prominent spot migrating on high voltage electrophoresis as phosphotyrosine shown in Fig. 6, lane *d*, was eluted with water, mixed with authentic phosphotyrosine, concentrated by lyophilisation and analysed by ascending chromatography on cellulose thin layer plates (Brinkman, CEL 300) in saturated ammonium sulphate: 1 M sodium acetate: isopropanol (40:9:1)[27]. The dashed line shows the position of the ninhydrin-stained phosphotyrosine internal marker.

Richert *et al.*[24] have argued that pp60src is more like a nucleotide kinase than a protein kinase, although the only demonstrated activity of pp60src is protein kinase activity. As described above, P120 of A-MuLV also has properties that suggest its autophosphorylation activity may represent formation of an enzyme intermediate, although we would not single out nucleotide kinases as the best parallel.

In summary, both the Rous sarcoma virus protein, pp60src, and the A-MuLV protein, P120, are membrane-associated, phosphorylating proteins that show rapid reactions at 0 °C (refs 22, 24). Early proteins of SV40 and polyoma, two other transforming viruses, also are phosphorylated *in vitro* and at least one of these seems to be phosphorylated at a tyrosine residue[20]. These similarities suggest that the leukaemogenic (and fibroblast transformation) activity of A-MuLV, the sarcomagenic activity of Rous sarcoma virus and the generalised transforming potential of DNA tumour viruses may have a similar basis.

This work was supported by grants from the American Cancer Society and the NIH. O.N.W. is a postdoctoral fellow of the Helen Hay Whitney Foundation. D.B. is an American Cancer Society Research Professor. Marker phosphorylated amino acids were from Dr J. Wang.

Note added in proof: T. Hunter and B. M. Sefton (*Proc. natn. Acad. Sci. U.S.A.*, in the press) and R. Erikson and colleagues (personal communication) have recently shown that the protein kinase activity associated with pp60src of Rous sarcoma virus specifically phosphorylates tyrosine residues.

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β-Chain contact sites in the haemoglobin S polymer

**R. L. Nagel[*], J. Johnson[*], R. M. Bookchin[*], M. C. Garel[†], J. Rosa[†], G. Schiliro[‡]
H. Wajcman[§], D. Labie[§], W. Moo-Penn[‖] & O. Castro[¶]**

* Division of Experimental Hematology, Department of Medicine, Albert Einstein College of Medicine, Bronx, New York 10461. † Institute National de la Santé et de la Recherche Medicale, Group U91 Creteil, France. ‡ Department of Pediatrics, University of Catania, Catania, Italy. § Institute National de la Santé et de la Recherche, Medical Group U15, Paris, France. ‖ Center for Disease Control, Atlanta, Georgia 30333. ¶ Department of Pediatrics, Howard University, Washington, DC 20060.

The amino acid residues involved in the areas of contact that stabilise the haemoglobin S polymer fibre seem to be the same ones that stabilise the basic unit of the deoxyhaemoglobin S crystal: the Wishner–Love double strand. The haemoglobin S fibre is probably formed by a unique packing of these double strands.

ANALYSIS of the supramolecular structure of the deoxyhaemoglobin (deoxy Hb) S polymer has been attempted by diffraction of high resolution electron microscopy plates[1] and by X-ray crystallography of aligned fibres[2]. A detailed description of the structure, however, must account for data about specific amino acid residues which alter the polymerisation. Our laboratory has approached this problem with a strategy that utilises binary mixtures of Hb S and Hb variants with mutations of residues at the molecular surface[3,4]. Since the predominant species in such mixtures are hybrid tetramers of the type $\alpha_2\beta^s\beta^x$ or $\alpha\beta^s\alpha^x\beta^A$, their behaviour can show whether the mutated residue affects the polymerisation of Hb S. This article describes several residues at the surface of the tetramer that appear to be involved in areas of contact in the polymer and compares these to the contacts found in deoxy Hb S crystals by Love and associates[5].

Isolation of mutants and other experimental procedures

Haemolysates were made from blood samples obtained after informed consent from donors with sickle cell anaemia (SS) diagnosed and characterised in our Heredity Clinic. These haemolysates were selected to contain less than 5% Hb F (measured as the alkaline-resistant fraction[6]) and were used without further purification. Hb Hope was obtained from a homozygous donor and contained less than 1% Hb F, and less than 2% Hb A_2 as measured by column chromatography[7].

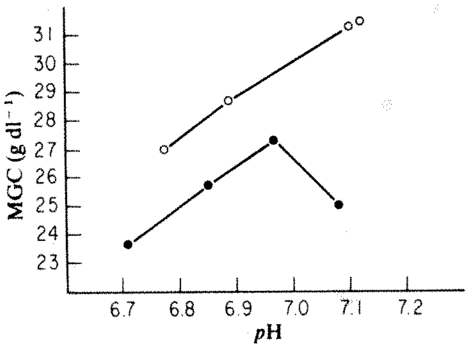

Fig. 1 Minimum gelling concentration (MGC) of Hb S (40%) + Hb A (60%) (○), and Hb S (40%) + Hb I Toulouse (60%) (●) at different pH. Hb I Toulouse when mixed with Hb S promotes gelation as compared to Hb A. Buffer: 0.15 M KPO_4, Temperature 24 °C.

The other haemoglobin variants used in these studies were isolated from haemolysates of heterozygotes' blood by chromatography on columns of DE-52 (Whatman) using 0.05 M Tris-HCl buffer with a pH between 7.5 and 8.2, depending on the haemoglobin, as described elsewhere in detail[4]. The characterisation of Hb Manhasset (β139 Asn → Lys) will be reported separately[8]. All samples were concentrated and simultaneously dialysed against 0.15 M potassium phosphate (pH 7.35) and minimum gelling concentrations were determined at 24 °C as described previously[9].

In some of the experiments we use dithionite to reduce methaemoglobin and/or to assure complete deoxygenation. The dithionite solution was prepared by mixing the powder anaerobically with 0.001 M NaOH. An appropriate aliquot of this solution was added anaerobically to the deoxygenated haemoglobin mixture. The final solution contained 1.2 M of dithionite per mol of haem and the final pH was consistently 6.9.

Rationale of the experimental design

We had previously proposed on the basis of indirect evidence[9] confirmed by others[10] that the two β-chains of Hb S behave unequally in the formation of the polymer. More recently, we obtained direct evidence[11] that only one of the β6 valine substitution sites of the Hb S tetramer is actively involved in an area of contact. The symmetrical area in the other β^s chain is not involved in any area of contact[12]. Due to the symmetry rela-

Table 1 Effect of β-chain mutations on the gelation of Hb S

Mutant	Site	Substitution	Hb S + Hb A* MGC (g dl⁻¹)	Hb S + Hb X MGC (g dl⁻¹)	ΔMGC	pH
J Baltimore	β16(A13)	Gly → Asp	29.0	32.5	+3.5	6.8†
J Amiens	β17(A14)	Lys → Gln	28.7	32.5	+4.0	6.9†
D Ouled Rabah	β19(B1)	Asn → Lys	32.10	35.3	+3.2	7.1
E	β26(B8)	Glu → Lys	32.4	33.2	—	7.1
J Lome	β59(E9)	Lys → Asn	32.3	33.1	—	7.1
			29.4	28.9	—	6.8†
I Toulouse	β66(E10)	Lys → Glu	31.5	25.0	−6.5	7.1
Pyrgos	β83(EF7)	Gly → Asp	32.1	35.1	+3.0	7.1
Agenogi	β90(F6)	Glu → Lys	32.7	33.7	—	7.2
Detroit	β95(FG2)	Lys → Asn	28.6	31.6	+3.0	6.9†
N Baltimore	β95(FG2)	Lys → Glu	32.0	38.5	+6.5	7.1
			29.5	37.0	+7.0	6.9†
Hofu	β126(H4)	Val → Glu	35.3	34.6	—	7.1
K Woolwich	β132(H10)	Lys → Gln	32.3	33.2	—	7.1
			29.2	29.8	—	6.8†
Hope	β136(H14)	Gly → Asp	32.4	32.7	—	7.1
Manhasset	β139(H17)	Asn → Lys	32.3	31.9	—	7.1

* Hb A was obtained from the same chromatographic separation in most cases.
† Deoxygenation with dithionite.

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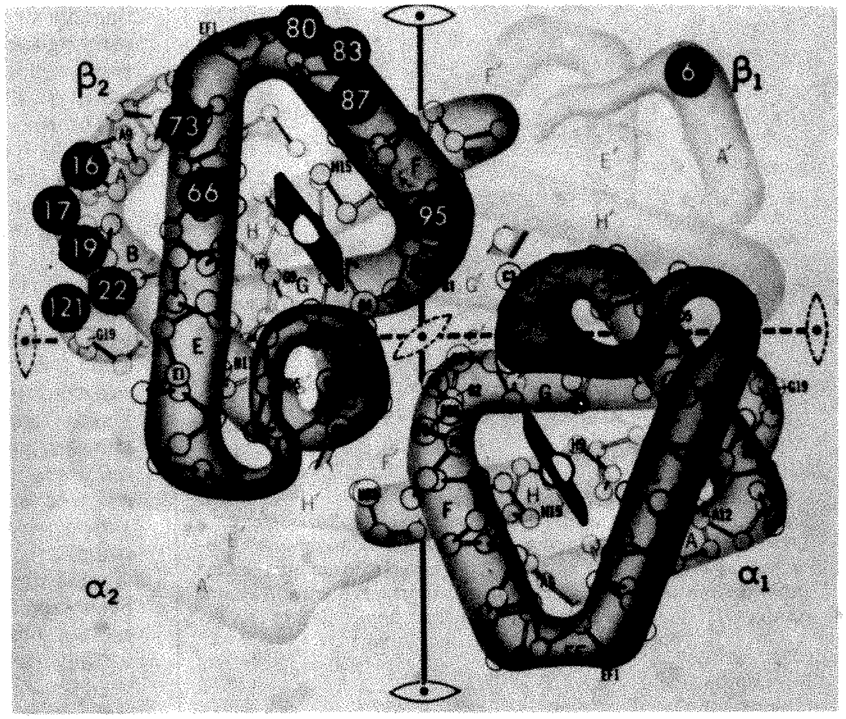

Fig. 2 Mutational sites (black circles with white numbers) that have been shown to interfere with the polymerisation of Hb S in solution. This diagram is a modification of a figure by Irving Geis. References for the mutations not reported here can be found in ref. 12. The diagram uses the nomenclature of Perutz for helical and nonhelical segments. White digits correspond to sequence numbers. Note that all mutational sites described correspond to the $\beta 2$ chain when $\beta 1$ is carrying the active 6-Val site.

tionships between the chains in the haemoglobin tetramer each β-chain has a different set of interactions with each of the α-chains. With one of the α-chains, a β-chain forms a highly stable system of strong hydrophobic and polar interactions resulting in a very stable $\alpha 1\beta 1$ dimer. The same $\beta 1$ chain forms weaker interactions with the $\alpha 2$ chain so that the $\alpha 1\beta 2$ interface is broken when the tetramer dissociates into dimers. These considerations highlight the need to define the effect of a surface residue specifically in terms of its activity in $\alpha 1$, $\alpha 2$, $\beta 1$, or $\beta 2$, in relation to the 'active' $\beta 6$ valine site.

To define β-chain interaction sites, we mix Hb S with the β-chain variant, Hb X (generally in the proportion of 40% Hb S and 60% Hb X). The predominant species in the mixture (because of the dimerisation of both parental tetramers and subsequent free rearrangement) is the hybrid $\alpha_2\beta^s\beta^x$. It is obvious that this allows us to measure the activity of the mutant residue located *trans* to the β-chain carrying the active $\beta 6$ Val substitution. In order to explore the effects of *cis* residues, Hb variants with two mutations in the same β-chains are required, one of which is identical to the Hb S substitution. Three such double mutants have been examined[3]. If the minimum gelling concentration of the doubly mutated Hb is abnormal, further information pertaining to the *cis* or *trans* character of the effect can be obtained by mixing this variant with Hb A and observing the behaviour of the hybrid $\alpha_2\beta^{s,x}\beta$.

Effect of several β mutations on Hb S polymerisation

The results obtained with the β-chain mutants are summarised in Table 1. Of the 14 mutants examined, 6 altered the polymerisation of Hb S. Mutations at sites $\beta 16$, $\beta 17$, $\beta 19$, $\beta 83$ and $\beta 95$ had an inhibitory effect, whereas the mutation at site $\beta 66$ facilitated polymerisation (Fig. 1), an effect similar to that found with substitutions at $\beta 121$ (Hb O Arab and Hb D Los Angeles)[13,14]. It should be emphasised that the assignment of these residues to areas of interaction pertain exclusively to the $\beta 2$ chain *trans* to the $\beta 1$ chain designated as the one carrying the active 6-Val site. The effects of the corresponding *cis* residues in the sequence of the $\beta 1$ chain remain to be defined. The magnitude of the alterations of gelation in mixtures with Hb S suggests that $\beta 66$ and the nearby $\beta 73$ site (previously studied)[15,16] is part of an important single area of interaction between the tetramers of the polymer.

Figure 2 shows all the β-chain sites that have been implicated in areas of contact, including the ones reported here. Those that have been examined and found thus far not to participate are listed in Table 2.

Assigned sites of interaction and the Hb S crystal

Wishner and his associates[5] have described the crystal structure of deoxy Hb S and found that the basic unit is a double strand stabilised with side-to-side and up-down interactions involving mainly the β-chains (Figs 3, 4). A salient feature of this structure is that only one of the β-chains has a 6-Val residue involved in a contact area. This is in accordance with our data on the formation of the Hb S polymer[12]. The relevance of this structure to the deoxy Hb S fibre can be tested further by determining if other contacts found in the crystal structure can be shown to be active in polymerising solutions of deoxy Hb S. Of the sites examined thus far, $\beta 66$, $\beta 73$, $\beta 83$ and $\beta 87$, which were found to be involved in the side-to-side contact of the crystal, were also

Table 2 Sites shown tentatively *not* to interfere with the polymerisation of Hb S in solutions

	Site	Substitution	Ref
β(2) trans	Deer Lodge	$\beta 2$ (His → Arg)	19
	G Makassar	$\beta 6$ (Glu → Ala)	19
	Leiden	$\beta 6$ (Glu → O)	20
	G San Jose	$\beta 7$ (Glu → Gly)	21
	Saki	$\beta 14$ (Leu → Pro)	22
	E	$\beta 26$ (Glu → Lys)	This article
	G Copenhangen	$\beta 47$ (Asp → Asn)	3
	C Ziguinchor	$\beta 58$ (Pro → Arg)	23
	J Lome	$\beta 59$ (Lys → Asn)	This article
	J Cairo	$\beta 65$ (Lys → Glu)	23
	J Chicago	$\beta 76$ (Ala → Asp)	3
	Agenogi	$\beta 90$ (Glu → Lys)	This article
	Alkylation	$\beta 93$ (Cys)	19
	Hofu	$\beta 126$ (Val → Glu)	This article
	Woolwich	$\beta 132$ (Lys → Gln)	This article
	Hope	$\beta 136$ (Gly → Asp)	This article
	Manhasset	$\beta 139$ (Asn → Lys)	This article
	S Travis	$\beta 142$ (Ala → Val)	24
β(1) cis	C Ziguinchor	$\beta 58$ (Pro → Arg)	23
	C Harlem	$\beta 73$ (Asp → Asn)	16
	S Travis	$\beta 142$ (Ala → Val)	24

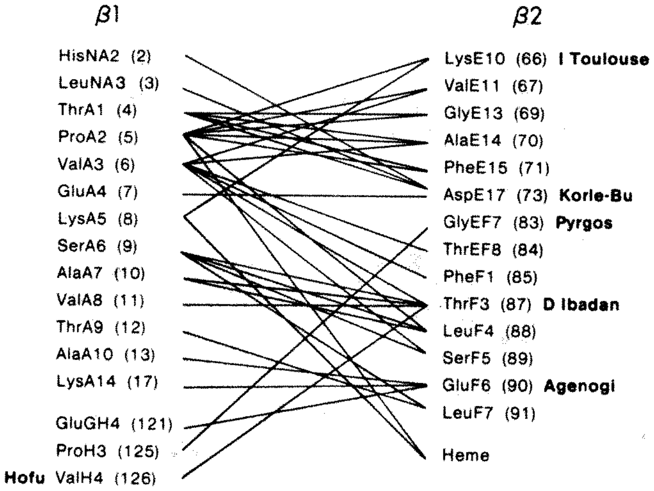

Fig. 3 Side-to-side contacts in the Wishner–Love double strand[5]. Lines connect residues within a 5 Å radius. Contiguous to the sequence site, we have indicated (in bold letters) the mutant used to explore that site. All the side-to-side interactions involve β-chain residues. Residues are identified by Perutz nomenclature and in parenthesis by the sequence number.

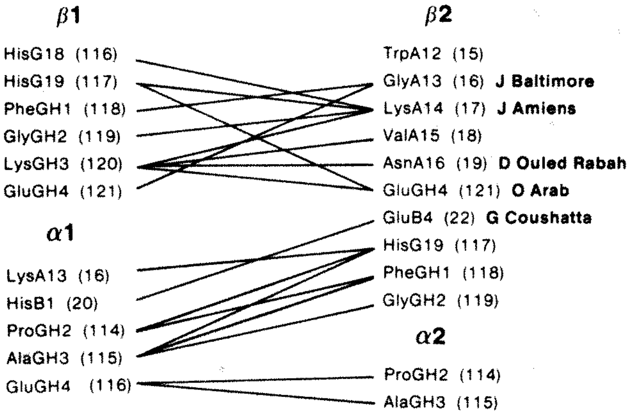

Fig. 4 Up-and-down contacts in the Wishner–Love double strand[5]. Lines connect residues within a 5 Å radius. Contiguous to the sequence site we have indicated (in bold letters) the mutant used to explore that site. Nomenclature as in Fig. 3.

found to have strong effects on the polymerisation of solutions of Hb S (Fig. 3). Note that these residues were shown to be part of the receptor site located in the β2 chain of the crystal (the β1 chain carries the 'active' β6-Val site). This, of course, is in complete agreement with our results, as our experimental design with Hb S–Hb X mixtures detects only the effects of the mutant β^x residues in the β2 chain. A further interesting point can be made with regard to the residue β126, that we have explored using Hb Hofu. Although β126-Val participates in the interacting site of the crystal it is provided by the same (β1) chain carrying the active 6-Val site. We do not find any effect of this residue, but this is not in contradiction with the crystal data, in which β2 126 is *not* in an area of contact.

Three residues are not coincident with the crystal data. Residue β80, implicated by the inhibitory effect of Hb Gifu[4], is not present in the side-to-side contact area, but it is very close, as demonstrated by the fact that residue β83 is involved. In addition, in the Hb F crystal, β80 is featured in this area of contact[17]. The residue at β90 is included in the side-to-side contact in the crystal, but we find that it is inactive in solution. It should be remembered, however, that this residue is at the edge of the contact area and interacting residues have been included using a radius of 5 Å (ref. 5) which is rather large relative to expected hydrogen bond distances and Van der Waals radii. Finally, residue β95, which we have examined using two mutants, is not involved at all in the areas of contact between the tetramers in the crystal double strand. This residue could be involved in other contacts that are specific for the fibre.

Now we turn to the up–down interaction along the crystal strand axis (Fig. 4). Of the residues involved by the crystallographic map of deoxy Hb S, β16, β17, β19, β22 and β121 have been examined. All of them have been found to be active in solution (Table 1, Fig. 2).

Conversely, of the 21 β-residues examined thus far that have been shown to be inactive in solution, 20 of them are not found in the areas of contact between the tetramers forming the double strands of the crystal (Table 2). The only exception was β90, as noted above.

In terms of the α-chain sites, there are only five contact sites provided by α1 and two contact sites provided by α2 that stabilise the up–down contacts of the double strand in the crystal. Of these, only α116 has been examined by Benesch *et al.*[18]; but until it is established whether the effect found is attributable to the α2 or α1 chain, this data cannot be used to test the crystal relevance to the solutions of Hb S.

Conclusions

The data presented here show remarkable coincidence between the crystallographically defined contact sites for the double strands of deoxy Hb S and the residues that appear to be active in solutions undergoing polymer formation. This suggests that the double strand is the building unit in the assembly of the fibre. At first approximation the supramolecular structure proposed by Dykes *et al.*[1] could be accommodated with seven double strands as defined in the crystal.

It should be kept in mind that the crystal and fibre cannot be identical structures. It is likely that residues of the α chain involved in the stabilisation between double strands in the crystal are not significant for the fibre. The stabilisation of the double strands present in the fibre may involve interactions between the α-chains which are not present in the crystal. The definition of these α–α contacts will provide the evidence required to establish the packing order of the Wishner–Love double strand in the haemoglobin S fibre.

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Abnormal expression of chromosomal rabbit β-globin gene in *Saccharomyces cerevisiae*

Jean D. Beggs*, **Johan van den Berg**†, **Albert van Ooyen** and **Charles Weissmann**

Institut für Molekularbiologie I, Universität Zürich, 8093 Zürich, Switzerland and *Plant Breeding Institute, Trumpington, Cambridge CB2 2LQ, UK

A 5.1 kilobase-pair segment of rabbit chromosomal β-globin DNA was joined to pJDB219, a plasmid consisting of pMB9, the 2-μ yeast plasmid and the yeast leu-2 *gene.* Saccharomyces cerevisiae *transformed with the globin DNA-containing hybrid produced β-globin-specific RNA. As compared to mature β-globin mRNA, these transcripts lacked* 20–40 *nucleotides from the 5′ end, contained all of the small intron and extended to about the middle of the large intron. Thus, no splicing of the primary β-globin transcript could be detected in the yeast cells.*

PROKARYOTIC cells are, in general, unlikely to provide an adequate environment for the spontaneous expression of chromosomal genes of higher eukaryotes. Besides the incompatibilities to be expected at the levels of RNA and protein synthesis, the existence within eukaryotic genes of intervening sequences (see ref. 1 for references), which are copied into primary transcripts and subsequently eliminated (see ref. 2 for references) by processes so far unknown in prokaryotes, suggests that only eukaryotic cells are promising hosts for testing the physiological expression of eukaryotic genes.

Yeast, as a lower eukaryote, is a possible candidate for such a test system. Vectors containing the yeast 2-μ plasmid are suitable for high frequency transformation of yeast[3–5], presumably because this plasmid replicates in yeast cells, yielding relatively high copy numbers and thus allowing maintenance without integration.

pJDB219, a hybrid DNA consisting of pMB9, the 2-μ yeast plasmid and the *leu*-2 gene of yeast, is maintained predominantly in plasmid form in yeast[3]. We have transformed *Saccharomyces cerevisiae* with a hybrid consisting of pJDB219 and a cloned rabbit chromosomal DNA segment including a complete β-globin gene with two intervening sequences and extended flanking regions[6,7]. We found that yeast thus transformed contained β-globin-specific transcripts 800–900 nucleotides long; however, these do not have normal termini and have not undergone splicing. An abstract of this work has been presented[8].

Transformation of yeast with cloned rabbit β-globin chromosomal DNA

A 5.1 kilobase-pair fragment of rabbit chromosomal DNA, containing a β-globin gene, has been cloned in plasmid pCRI (Z-pCRI/RchrβG-1) and characterised by restriction analysis[6]; the nucleotide sequence of the gene and its flanking regions was

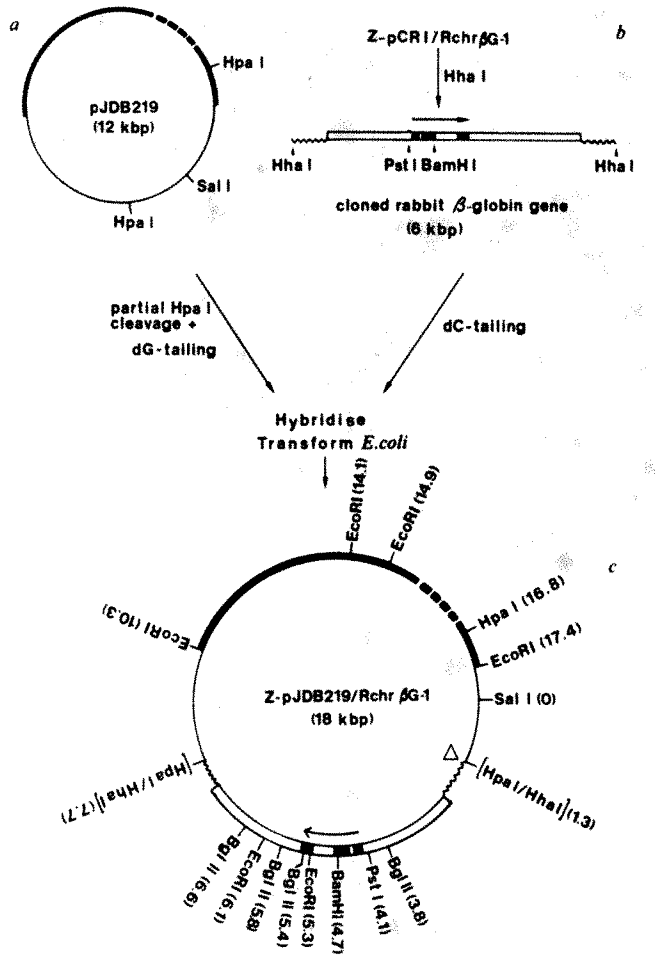

Fig. 1 Construction of hybrids consisting of rabbit chromosomal β-globin DNA, the 2-μ yeast plasmid, the yeast *leu*-2 gene and plasmid pMB9. The hybrid plasmid pJDB219 (*a*), consisting of pMB9 (thin lines), the 2-μ yeast plasmid (thick lines) and the *leu*-2 gene (dashed line) was partially digested with *Hpa*I, the fragments were separated on a 0.6% agarose gel and full-length linear DNA (12.4 kilobases) was extracted, purified on DEAE-cellulose[14], digested with λ exonuclease and elongated with dGMP residues using terminal transferase[22]. The chromosomal β-globin plasmid Z-pCRI/RchrβG-1 (ref. 6) was cleaved with *Hha*I, the globin-DNA-containing 6.4-kilobase fragment (*b*) purified by sucrose gradient centrifugation and elongated with dCMP residues[22]. The elongated plasmid and globin DNAs (40 ng and 10 ng, respectively) were hybridised in 50 μl 50 mM Tris-HCl (*p*H 7.6), 100 mM NaCl, 5 mM EDTA for 1 h each at 65 °C, 46 °C and 37 °C, and used to transform *E. coli* χ1776 (ref. 6). Eight tetracycline-resistant clones were obtained and screened by the Grunstein–Hogness[9] procedure using ^{32}P-labelled β-globin cDNA as probe[19]. Two plasmids, Z-pJDB219/RchrβG-1 and -3 (abbreviated to YRβG-1 and YRβG-3), characterised by restriction analyses as shown in Fig. 2, were recloned in *E. coli* HB101 and used for further work. Both plasmids had the globin insert in the orientation shown (*c*); YRβG-3 has a 1,400 base pair deletion at the position indicated by Δ.

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† Present address: Gist-Brocades, Postbus 1, Delft, Holland. ‡ Present address: Imperial College of Science and Technology, London SW7 2AZ, UK.

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determined by van Ooyen *et al.*[7]. The rabbit DNA sequence (with pCRI-derived flanking segments) (see Fig. 1) was excised with *Hha*I and elongated with dCMP residues at the 3′ termini. pJDB219, which contains two *Hpa*I sites, one in the 2-μ yeast plasmid region, the other within the pMB9 moiety, was partially cleaved with *Hpa*I, the full-length linearised molecules were isolated, and elongated with dGMP residues (see Fig. 1). *Escherichia coli* χ1776 was transformed with the hybridised mixture of the two DNAs, and tetracycline-resistant clones containing the globin sequence were identified by the Grunstein–Hogness[9] procedure, using ^{32}P-labelled cloned β-globin cDNA as probe. Hybrid plasmids from two colonies were recloned in *E. coli* HB101, because the original clones contained a minor species of plasmid without a globin insert. Two isolates, Z-pJDB219/RchrβG-1 and Z-pJDB219/RchrβG-3 (abbreviated to YRβG-1 and YRβG-3), contained the β-globin *Hha*I fragment in the *Hpa*I site of the pMB9 moiety in the orientation shown in Fig. 1. The two hybrid plasmids were of different size. As shown by restriction analysis (Fig. 2), YRβG-3 contained a deletion of about 1.4 kilobases at or near the junction between the plasmid and the 5′ proximal part of the globin DNA insert. Both hybrids contained the complete globin-specific DNA, as shown by the presence of the 1.7 and 0.4 kilobase *Bgl*II fragments.

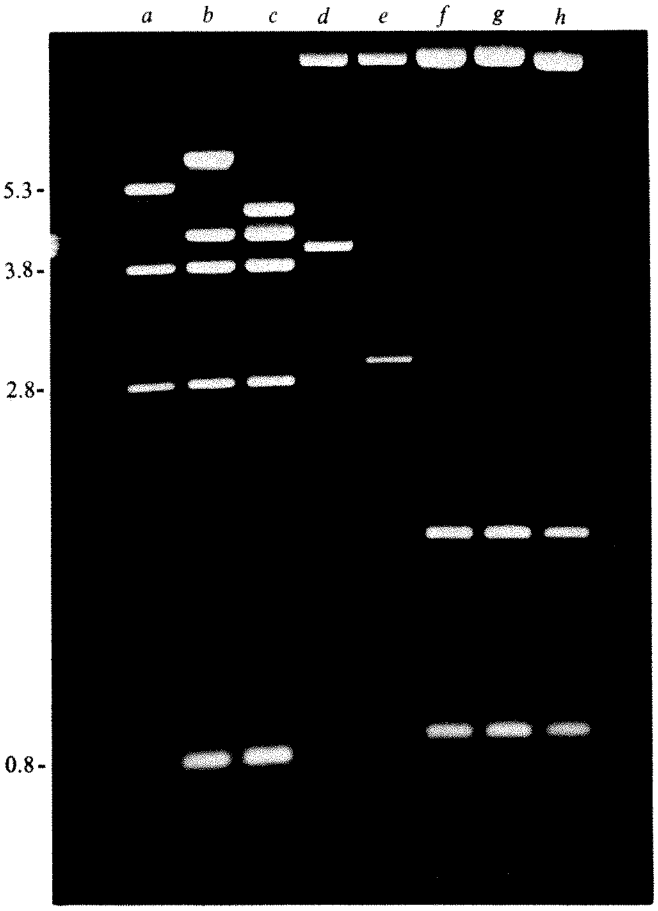


Fig. 2 Restriction site analysis of hybrid plasmids YRβG-1 and YRβG-3, containing pMB9, the 2-μ yeast plasmid, the yeast *leu*-2 gene and a rabbit β-globin chromosomal DNA segment. Plasmid DNA was prepared from transformed *E. coli* HB101, by lysing bacteria with SDS[23] or Triton X-100 (ref. 24), centrifuging, and separating linear and circular DNA by the procedure of Currier and Nester[25], as outlined in Wilkie *et al.*[26]. DNA was digested with the restriction enzymes indicated below and the products analysed by electrophoresis through a 1% agarose gel in 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA (*p*H 8.2). The gels were stained with ethidium bromide and photographed in UV light. *a*, *b* and *c*, *Eco*RI-digested pJDB219 (ref. 3) (*a*), YRβG-1 (*b*) and YRβG-3 (*c*); *d*, YRβG-1 and *e*, YRβG-3, both doubly digested with *Sal*I and *Pst*I; *f*, *g* and *h*, *Bgl*II-digested Z-pCRI/RchrβG-1 (*f*), YRβG-1 (*g*) and YRβG-3 (*h*). The orientation of the inserts was deduced from the sizes of the two largest fragments obtained by digestion with *Eco*RI (*b*) and (*c*), or *Sal*I and *Pst*I (*d*) and (*e*) (compare Fig. 1), and was confirmed by double digestion with *Eco*RI and *Pst*I or *Eco*RI and *Sal*I (not shown).

S. cerevisiae MC16 leu$^-$ (ref. 3) was transformed with YRβG-1 and YRβG-3 isolated from *E. coli* χ1776 or *E. coli* HB101, and leucine-independent colonies were isolated. One yeast clone from each transformation experiment was examined in greater detail. The results described below were the same regardless of whether YRβG-1 or -3 was used, or whether the plasmid had been recloned or not. Total DNA from the transformed yeast clones, as well as the original hybrid plasmid DNAs were digested with *Pst*I and *Bgl*II, and subjected to agarose gel electrophoresis. The DNA fragments were transferred to nitrocellulose filters[10] and hybridised to ^{32}P-labelled cloned β-globin cDNA. As shown in the autoradiogram of Fig. 3, the pattern of globin-specific bands given by the transformed yeast DNA was the same as that of the original β-globin DNA -containing plasmid Z-pCRI/RchrβG-1. From the intensity of the bands relative to those of known amounts of β-globin DNA we estimate that there were multiple copies of globin DNA per cell. Transfer of uncleaved DNA from transformed yeast according to Southern[10] followed by hybridisation with ^{32}P-labelled, cloned β-globin cDNA gave several bands; most preparations lacked DNA with the mobility of YRβG-1 or YRβG-3. Thus, the hybrid plasmid had been rearranged in the yeast; however, the globin-specific sequence was not changed, as shown by Fig. 3.

Characterisation of β-globin-specific transcripts in yeast

To determine whether globin-specific RNA had been synthesised in the transformed yeast cells, total RNA was subjected to agarose gel electrophoresis in denaturing conditions, transferred to diazobenzyloxymethyl paper[11], and hybridised with ^{32}P-labelled, cloned β-globin cDNA. As shown in Fig. 4, a strong β-globin-specific band with a mobility corresponding to an RNA of 800–900 nucleotides was found in yeast cells transformed with either YRβG-1 or YRβG-3. This RNA is thus longer than mature rabbit β-globin mRNA, about 700 nucleotides[12], but shorter than the 15S β-globin precursor RNA, about 1,450 nucleotides[13], including the poly(A) tails in both cases. By comparing the intensity of the globin-specific RNA band with that of known amounts of β-globin mRNA, we estimate that about 0.01% of the total yeast RNA was β-globin-specific.

The β-globin-specific RNA was further characterised by a modification[14,15] of the Berk–Sharp[16] hybridisation technique. To map the 5′ terminus of the transcript, a 223 base-pair restriction fragment of the cloned β-globin chromosomal DNA extending from the *Mbo*II cleavage site within the first exon (position 128) to the *Pst* site 95 nucleotides upstream from the beginning of the mRNA[7], labelled at the 5′ terminus of the minus strand (*Mbo*II cleavage site), was prepared. This probe was denatured and hybridised with the RNA sample in 80% formamide. After digestion with endonuclease S$_1$, the sample was denatured and the labelled DNA sized by polyacrylamide gel electrophoresis in 7 M urea. A radioactive DNA fragment is recovered if the labelled 5′ terminus of the DNA probe was hybridised to the RNA; its length is a measure for the distance between the *Mbo*II site and the 5′ terminus of the RNA or the beginning of extended mismatching between the RNA and the probe. As shown in Fig. 5*A*, natural rabbit β-globin mRNA protected a DNA fragment about 128 nucleotides in length; the DNA protected by RNA from β-globin transformed yeast cells gave five major bands, corresponding to 88, 91, 93, 102 and 110 nucleotides. The β-globin-specific transcripts from yeast apparently have several 5′ termini, all of which map downstream from the position corresponding to the 5′ terminus of natural β-globin mRNA. The 223-nucleotide band corresponds to the fully-protected probe and is believed to be due to self-annealing of the DNA; the presence of transcripts originating upstream, beyond the *Pst*I site, cannot be excluded.

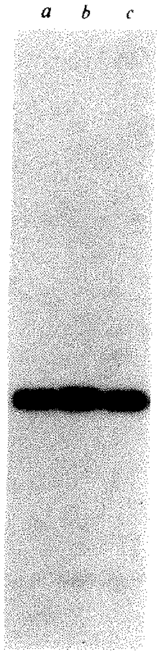


Fig. 3 Demonstration of β-globin-specific sequences in DNA from *S. cerevisiae* transformed with the β-globin DNA-containing hybrid plasmids YRβG-1 and YRβG-3. *S. cerevisiae* MC16αleu2-3 his4-712^{FS}ade2-1lys2-1SUF2 (G. R. Fink) was converted to spheroplasts and transformed with YRβG-1 and YRβG-3 DNA as described elsewhere[3]. Clones of transformed yeast were selected on plates lacking leucine[3]. One clone from each transformation experiment (Mc16/YRβG-1 and MC16/YRβG-3) was selected for further analysis. Transformed yeast was grown to late log phase in minimal medium without leucine (0.67% Difco yeast nitrogen base without amino acids, 2% glucose, 20 mg l^{-1} adenine, lysine and histidine). The cells were converted to spheroplasts[3] and DNA was isolated[27]. DNA was digested with *Pst*I and *Bgl*II simultaneously, subjected to electrophoresis through a 1% agarose gel in 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA (*p*H 8.2) and transferred to a Millipore membrane[10]. Globin DNA sequences were detected by hybridisation with the cloned rabbit β-globin cDNA fragment described earlier[19], ^{32}P-labelled by nick-translation[28] (specific activity, 3.5 × 10^{7} c.p.m. μg^{-1}); (*a*) Z-pCRI/RchrβG-1, (*b*) YRβG-1 and (*c*) YRβG-3.

To determine whether the small intron was present in the β-globin transcript, a hybridisation experiment similar to the one above was carried out, using as probe the 4 kilobase-pair *Bam-Sal* fragment of Z-pCRI/RchrβG-1, labelled at the 5′ (minus strand) terminus of the *Bam* cleavage site. As shown in the scheme of Fig. 6, an RNA molecule lacking the small intron would protect the probe from the 5′-^{32}P-labelled *Bam* site to the position where the sequences of β-globin mRNA and chromosomal DNA no longer match (at the small intron), yielding a fragment of 212 nucleotides[7]. If the small intron were present, the probe would be protected up to the region corresponding to the 5′ end(s) of the transcripts, yielding fragments between 440 and 462 nucleotides. The autoradiogram of Fig. 6 shows the presence of a band at a position corresponding to about 440 nucleotides length, but no signal at the position corresponding to 212 nucleotides, proving that the small intron was present in the β-globin-specific transcripts.

Finally, the 3′ termini of the transcripts were mapped using the 14 kilobase-pair *Bam-Sal* fragment of the β-globin chromosomal DNA plasmid Z-pCRI/RchrβG-1 (ref. 6) labelled at the 3′ (minus strand) terminus of the *Bam* site. This probe was prepared by filling up the termini of the *Bam*-cleaved β-globin chromosomal plasmid with α-^{32}P-deoxynucleotides using DNA polymerase I, cleaving with *Sal*I and isolating the 14 kilobase-pair fragment. Preliminary experiments showed that despite hybridisation in 80% formamide, which disfavours DNA–DNA reannealing[17], a band corresponding to the full-length probe was found whether RNA was present or not. Therefore, to preclude protection of the labelled 3′ terminus by self-annealing, the *Bam* fragment was subjected to digestion with 5′λ exonuclease, sufficient to remove about 2,000 nucleotides, before cleavage with *Sal*I. Using this probe, hybridisation with RNA from yeast transformed with YRβG-1 and YRβG-3 yielded four major bands of 270, 295, 340 and 350 nucleotides (Fig. 5*B*). As the distance from the (filled-up) *Bam* site to the 5′ border of the large intron is 18 nucleotides, the majority of the β-globin transcripts extends to about the middle of the large intron. Because, an 18-nucleotide fragment could not be detected for authentic mRNA in the conditions of this experiment, we cannot exclude the possibility that some transcripts had undergone splicing; however, because most transcripts were 800–900 nucleotides long (see Fig. 4), spliced transcripts could only constitute a minority of the population.

To determine whether the β-globin-specific transcripts were polyadenylated, yeast RNA was fractionated on oligo(dT)-cellulose, and flow-through and retained fractions were assayed for the presence of globin-specific sequences either by the transfer procedure of Alwine *et al.*[11] or by the modified Berk–Sharp method[14]. The results were variable; even with the same RNA preparation in some experiments 30%, in others 70% of

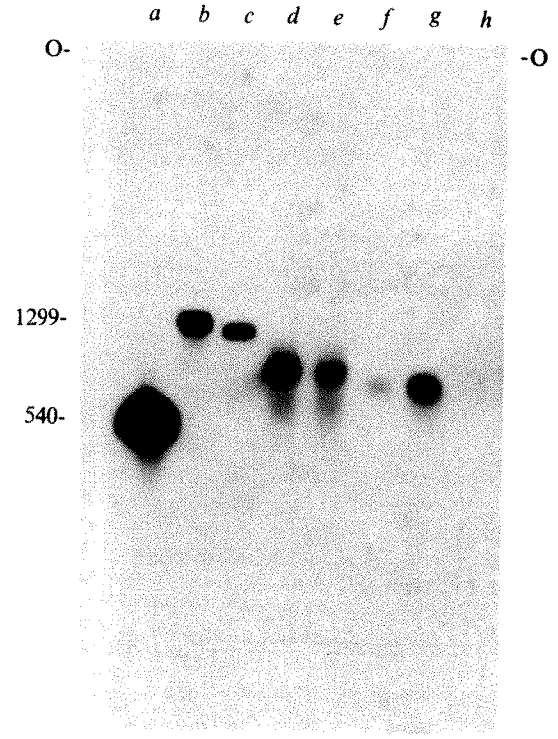


Fig. 4 The electrophoretic mobility of β-globin-specific RNA from *S. cerevisiae* transformed with the rabbit β-globin DNA-containing hybrid plasmid YRβG-1 and -3. *S. cerevisiae* was grown as described in the legend to Fig. 3. The cells were collected by centrifugation, washed in water, and suspended in 2 ml (per g of wet weight) 0.32 M sucrose, 20 mM Tris-HCl (*p*H 7.5), 10 mM EDTA containing heparin at 0.5 mg ml^{-1}. One volume of acid-washed glass beads was added and the sample was shaken on a vortex mixer for 1 min at top speed. The extract was diluted with 10 volumes 50 mM Tris-HCl (*p*H 7.5), 100 mM NaCl, 5 mM EDTA, 0.5 mg ml^{-1} heparin, 1% SDS, extracted with phenol and chloroform, and precipitated with ethanol. 24 μg RNA were recovered per ml culture. RNA samples were treated with glyoxal in dimethylsulphoxide at 50 °C for 60 min[29], subjected to electrophoresis through a 1.5% agarose gel in 10 mM sodium phosphate (*p*H 7), and transferred to diazobenzyloxymethyl paper[11]. β-Globin DNA-specific sequences were visualised by hybridisation in the conditions of Alwine *et al.*[11] with the cloned rabbit β-globin cDNA described earlier (ref. 19 and legend to Fig. 2), ^{32}P-labelled to 1.6 × 10^{8} c.p.m. μg^{-1} by nick translation with [α-^{32}P]dATP and [α-^{32}P]dCTP (400 Ci mmol^{-1} each, NEN). Denatured restriction fragments of rabbit β-globin-specific DNA and rabbit β-globin mRNA were used as size markers. *a*, Cloned rabbit β-globin cDNA[19]; *b* and *c*, plasmid Z-pCRI/RchrβG-1 digested with *Pst*I and *Bgl*II (1,299 base pairs) (*b*) or *Pvu*II and *Bgl*II (1,209 base pairs) (*c*); *d* and *e*, RNA from *S. cerevisiae* MC16 transformed with YRβG-1 (*d*) or YRβG-3 (*e*); *f* and *g*, rabbit (α + β) globin mRNA 2 ng (*f*) or 10 ng (*g*); *h*, RNA from *S. cerevisiae* MC16 transformed with pJDB219.

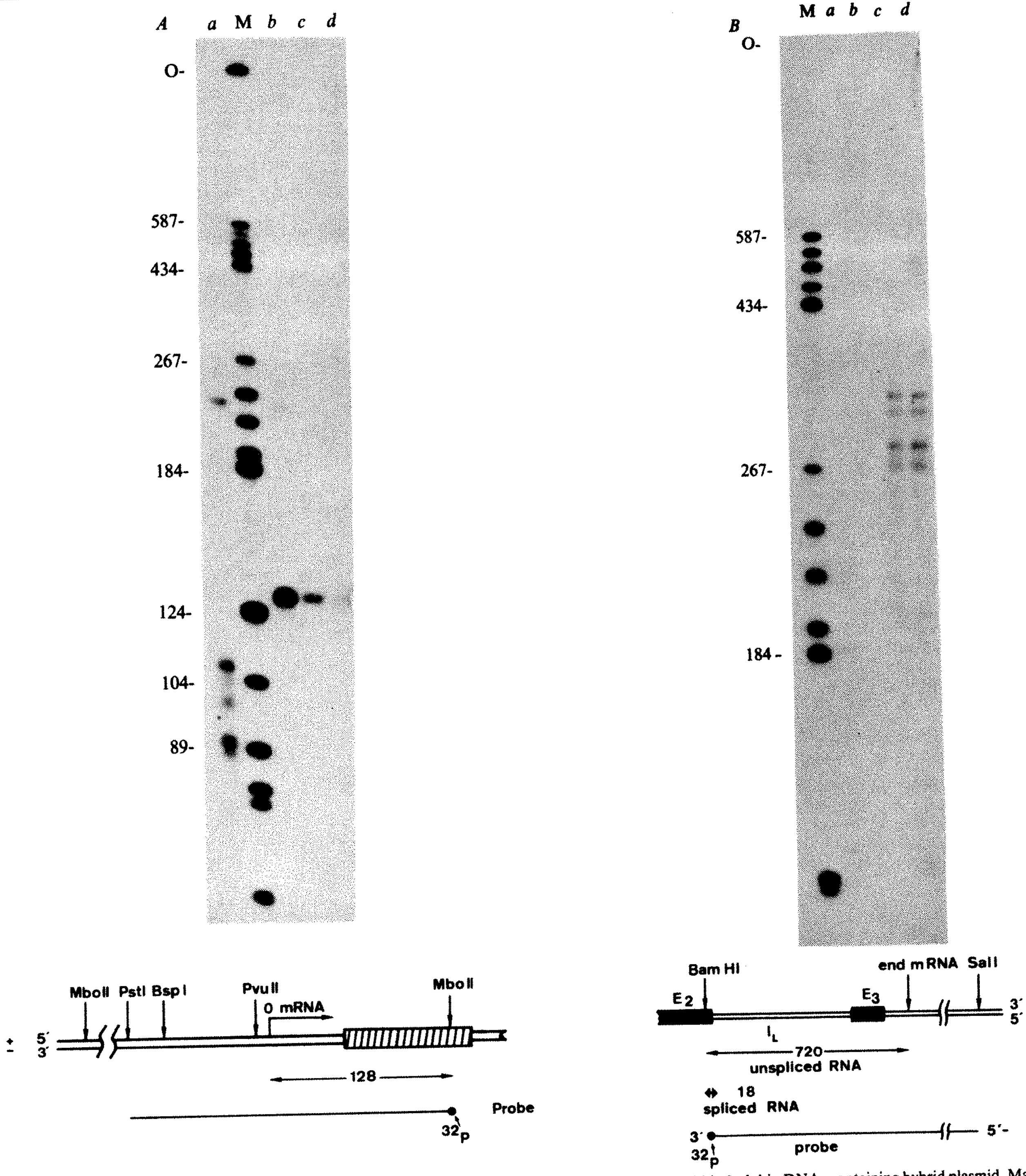


Fig. 5 Mapping of the termini of the β-globin-specific RNA from *S. cerevisiae* transformed with a rabbit β-globin DNA -containing hybrid plasmid. Mapping was carried out by a modification[14] of the Berk–Sharp[16] procedure. In this procedure a denatured, terminally ^{32}P-labelled DNA restriction fragment is hybridised to the RNA samples, treated with S_1 nuclease, and the length of the protected ^{32}P-labelled fragment is determined by polyacrylamide gel electrophoresis in 7 M urea. RNA from *S. cerevisiae* MC16/YRβG-1 was obtained as described in the legend to Fig. 4. *A*, Mapping of the 5′ termini. The DNA probe (see bottom section of figure) was prepared as follows. Plasmid Z-pCRI/RchrβG-1 was digested with *Hha*I, the 6.4 kilobase-pair fragment[6] isolated by sucrose gradient centrifugation, cleaved with *Mbo*II and 5′-terminally labelled with [γ-^{32}P]ATP[30] (specific activity, 3200 Ci mmol^{-1}). After cleavage with *Pst*I, the 223 base-pair fragment was isolated by electrophoresis through and extraction[14] from a 1.8% agarose gel in 89 mM Tris-borate (*p*H 8.2), 2.5 mM EDTA, ethidium bromide 1 μg ml^{-1}. The hybridisation reaction contained in 10 μl of 80% w/v formamide, 0.4 M NaCl, 0.04 M PIPES (*p*H 6.4), 1 mM EDTA, 11 ng denatured probe (about 7,000 d.p.m.) and RNA as indicated. Hybridisation was for 16 h at 57 °C under paraffin oil. The samples were digested with S_1 nuclease, extracted with phenol chloroform 1:1, and further processed as described elsewhere. Electrophoresis was in 89 mM Tris-borate (*p*H 8.2), 2.5 mM EDTA, 7 M urea on 5% polyacrylamide gels for 4 h at 700 V. Autoradiography was with use of Ilford fast tungstate intensifying screens, for 3 d at −70 °C. *a*, RNA (10 μg) from MC16 yeast transformed with YRβG-3; *b*–*d*, 3, 1 and 0.3 ng rabbit (α + β)-globin mRNA; M, marker (pBR322 cleaved with BspI and 5′-^{32}P-labelled). O, mRNA, position corresponding to origin of mRNA, hatched box, first segment of coding sequence. *B*, Mapping of the 3′ termini. The experiment is similar to that described above, but the probe was labelled at the 3′ terminus of the minus strand to permit downstream mapping (see bottom section of figure). Z-pCRI/RchrβG-1 (4 μg), cleaved with *Bam*HI, was incubated in 100 μl of 70 mM potassium phosphate buffer (*p*H 6.9), 100 mM NaCl, 5 mM $MgCl_2$ and 10 mM dithiothreitol with 3 units of DNA polymerase I (Boehringer Mannheim) and 5 μM each dTTP and dGTP, and 0.14 μM each [α-^{32}P]dATP and [α-^{32}P]dCTP (2 Ci mmol^{-1}, NEN) to label the 3′ termini. The labelled DNA was treated with *Sal*I and the larger (14 kilobase pairs) fragment, which contains the 3′ proximal segment of the β-globin DNA (and the ^{32}P-labelled minus strand 3′ terminus) was isolated by electrophoresis through a 0.7% agarose gel. To reduce the background resulting from self-annealing of the flush ends of the probe, the ^{32}P-labelled fragment (1 μg) was digested with 1 unit of 5′λ exonuclease[31,32] in 50 μl of 50 mM Na-cacodylate (*p*H 9.5), 5 mM $MgCl_2$, 50 mg ml^{-1} bovine serum albumen for 20 min at 23 °C. Each hybridisation assay (10 μl) contained 20 ng of denatured probe (20,000 c.p.m.) and RNA as indicated. The samples were processed as described above however, hybridisation was at 37 °C. Yeast RNA was prepared as described in the legend to Fig. 4. M, marker pBR322 cleaved with *Bsp*I and [5′-^{32}P]-labelled; *a* and *b*, 4 and 1 ng (α + β)-globin mRNA; *c* and *d*, RNA from *S. cerevisiae* MC16 transformed with YRβG-1 (*c*) or YRβG-3 (*d*). E_2 and E_3, second and third segments of coding sequence; I_L, large intervening sequence.

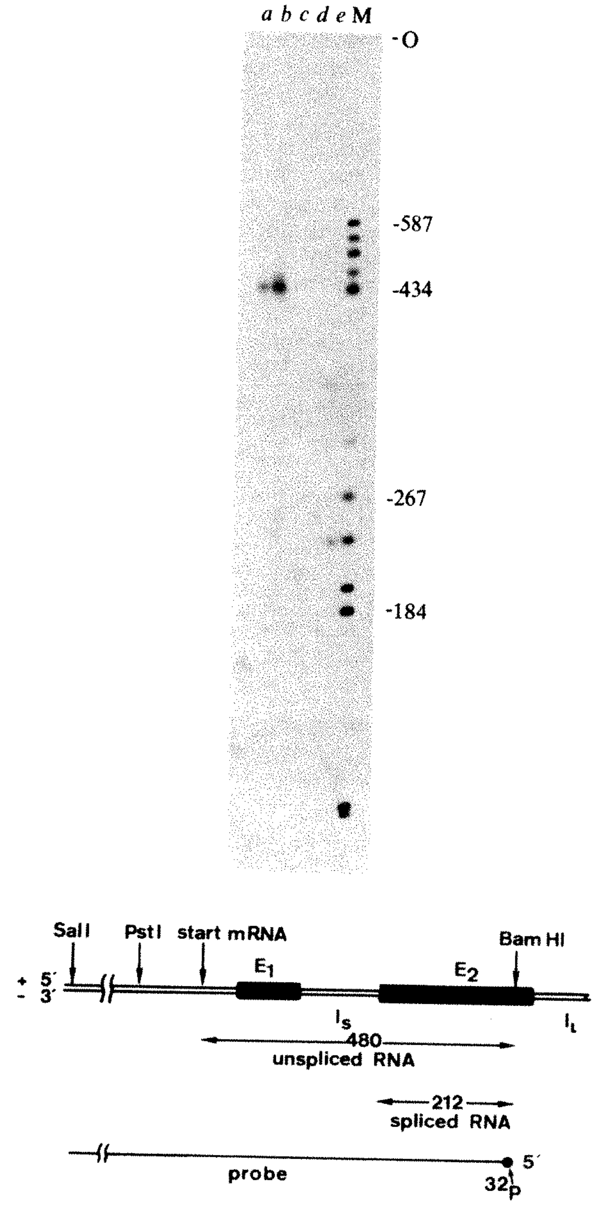
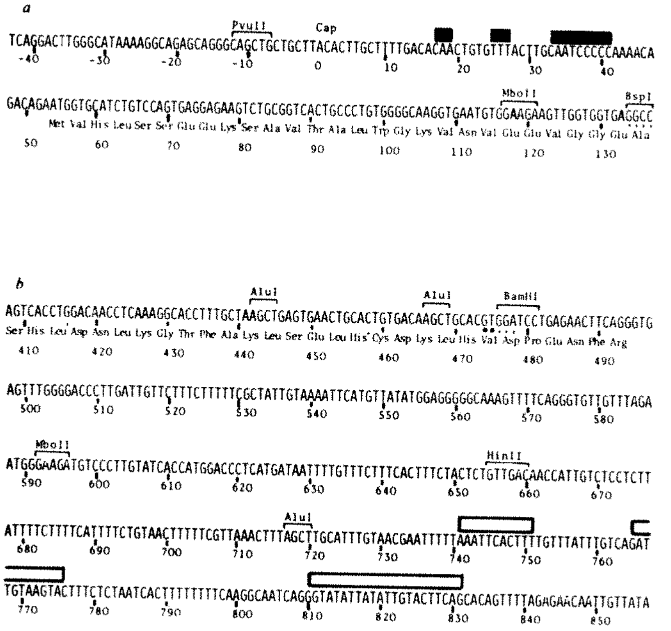


Fig. 6 Demonstration of the presence of the short intervening sequence in β-globin-specific transcripts from *S. cerevisiae* transformed with the rabbit β-globin DNA-containing hybrid plasmids YRβG-1 and YRβG-3. Mapping was carried out by the modified Berk–Sharp procedure[14] as outlined in the legend to Fig. 5 and the bottom part of this figure. Yeast RNA was prepared as described in the legend to Fig. 4. The DNA probe was prepared by cleaving Z-pCRI/RchrβG-1 with *Bam*HI, labelling the 5′ termini with [γ-^{32}P]ATP (3200 Ci mmol^{-1})[30] and cleaving with *Sal*I. The 4 kilobase-pair *Bam-Sal* fragment was isolated by electrophoresis through a 0.7% agarose gel. Hybridisation mixtures (10 µl) contained 50 ng of probe (20,000 c.p.m.) and RNA as indicated. The samples were processed as described in the legend to Fig. 5 and analysed by electrophoresis through a 3.5% polyacrylamide gel in 7 M urea. M, marker (pBR322 DNA cleaved with *Bsp*I and 5′ terminally ^{32}P-labelled); *a–c*, 10 µg RNA from *S. cerevisiae* MC16 transformed with YRβG-3 (*a*), YRβG-1 (*b*) and pJDB219 (*c*); *d* and *e*, rabbit ($\alpha+\beta$)-globin mRNA, 1 and 4 ng, respectively. E$_1$ and E$_2$, first and second segment of coding sequence; I$_s$, small intervening sequence.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MboII
BspI
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Met Val His Leu Ser Ser Glu Glu Lys Ser Ala Val Thr Ala Leu Trp Gly Lys Val Asn Val Glu Glu Val Gly Gly Glu Ala
50 60 70 80 90 100 110 120 130
b
AluI
AluI
BamHI
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Ser His Leu Asp Asn Leu Lys Gly Thr Phe Ala Lys Leu Ser Glu Leu His Cys Asp Lys Leu His Val Asp Pro Glu Asn Phe Arg
410 420 430 440 450 460 470 480 490
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MboII
HinII
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AluI
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TGTAAGTACTTTCTCTAATCACTTTTTTTTCAAGGCAATCAGGGTATATTATATTGTACTTCAGCACAGTTTTAGAGAACAATTGTTATA
770 780 790 800 810 820 830 840 850

Fig. 7 Approximate map positions of the 5′ and 3′ termini of rabbit β-globin transcripts in yeast. The nucleotide sequence and numbering are taken from Van Ooyen *et al.*[7]. Position 1 corresponds to the capped nucleotide. The 5′ and 3′ termini of the β-globin transcripts map in the areas indicated by black and open boxes, respectively. *a*, Sequences around the beginning of the β-globin gene. *b*, Sequences around the 5′ end of the large intron.

the globin-specific transcripts were in the retained fraction, in conditions where authentic β-globin mRNA was retained completely. This may mean that the chain length of poly(A), when present, was too short to ensure stable binding to the oligo(dT) column. A poly(A) tail of less than 15 nucleotides length appears to be insufficient to bind to oligo(dT)-cellulose[18].

Discussion

RNA from yeast cells transformed with the β-globin DNA-containing hybrid plasmids was intermediate in size between mature β-globin RNA and the 15S β-globin precursor. The β-globin-specific transcripts from yeast were about 20–40 nucleotides shorter at the 5′ end than normal globin mRNA (Fig. 7), contained the small intron and extended into the first half of the large intron where they terminated in an AT-rich region around the 250th to the 330th nucleotides of the large intron (Fig. 7), apparently heterogeneously and for the most part without a poly(A) appendage long enough to allow binding to oligo(dT)-cellulose. This should be considered in the light of the finding that the poly(A) tails of yeast mRNAs are on average considerably shorter than those of animal cell mRNAs; 50% of all yeast RNA molecules isolated on oligo(dT) cellulose have poly(A) chains less than 32 nucleotides in length[18]. It is not *a priori* certain that the 3′ and 5′ ends, as we have mapped them, correspond to the authentic termini of the transcripts. For one, the S$_1$ treatment may have trimmed the non-labelled end of the hybridised probe, particularly if it maps in an AT-rich region. However, S$_1$ digestions carried out in different conditions yielded similar patterns (R. Weaver, unpublished data) and thus do not support this hypothesis. Another possibility is that the primary transcript contained non-globin plasmid sequences at one or both ends and that a yeast-specific splicing reaction excised RNA segments comprising the junction to the globin sequence. The resulting structure would preclude detection of termini by the modified Berk–Sharp assay with a globin-specific probe. However, the length of the transcript, about 800–900 nucleotides, as estimated by its electrophoretic mobility, was in fairly good agreement with the length obtained by adding up the segments mapped upstream and downstream from the *Bam* site by the modified Berk–Sharp procedure, namely about 790 nucleotides, suggesting that no substantial segments of the transcripts escaped detection.

It is not clear whether the abnormal 5′ termini of the β-globin-specific RNAs from yeast represent transcription initiation sites recognised by a yeast RNA polymerase, or whether they result from the processing or degradation of longer transcripts originating at the true promoter site or even further upstream. Similarly, the 3′ termini may represent erroneous termination of transcription in T-rich regions or termini resulting from degradation or processing.

Why are the globin transcripts recovered from yeast not spliced? Possibly, yeast does not have a splicing system capable of processing the rabbit β-globin transcript. Alternatively, the transcripts and the appropriate splicing system are not present in the same compartment. Less likely, the β-globin transcript formed in yeast may not be susceptible to splicing because it lacks part of the 5′ terminal and more than a third of the 3′ proximal segment; perhaps a complete transcript is required to allow the formation of a secondary or tertiary structure essential for the splicing process.

In any event, the highly heterologous system we have described is obviously inadequate for the study of transcription and processing of the β-globin gene, which in more homologous systems, such as mouse L cells transformed with the rabbit β-globin gene[19], or monkey cells infected with β-globin–SV40 hybrid DNA[20,21] has proven capable of giving rise to normal β-globin mRNA.

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LETTERS

The composition of the Trojan asteroids

Jonathan Gradie & Joseph Veverka

Laboratory for Planetary Studies, Cornell University, Ithaca,
New York 14853

Physical studies have shown that most asteroids are covered with material of low visual albedo (p_v~0.04) having flat, nearly featureless reflection spectra between 0.4 and 1.1 µm (ref. 1). This large class of objects, the C asteroids, accounts for a progressively larger fraction of minor planets as one goes from the inner to the outer regions of the asteroid belt, a trend which has been interpreted as evidence of a decrease in effective condensation temperature in the original solar nebula with increasing distance from the proto-Sun[2]. We suggest here that the very low albedos and red spectra of some Trojan asteroids can be explained by the presence of kerogen-like organic compounds. Materials containing these types of carbonaceous substances, rather than those found in the more familiar carbonaceous chondrite matrix, may have been the primary rocky condensate in the outer Solar System and may therefore be typical of the rocky component of comet nuclei.

A widely accepted interpretation of these dark, spectrally neutral materials covering the asteroids, is that they consist of silicate clay minerals (montmorillonite, chlorite, serpentine) intermixed with opaque materials such as magnetite and finely divided carbon-rich compounds. Thus, the surface materials of the C asteroids are considered to be closely similar to, and in some cases perhaps identical with, the carbonaceous chondrite meteorites. Mixtures of small amounts of magnetite and carbon black with montmorillonite can be made to match both the albedo and the general spectral reflectance of some carbonaceous chondrites, although the carbonaceous matrix material in such meteorites is much more complicated in composition than carbon black[4,5]. The spectral reflectance curves (normalised at 0.56 µm) of the 115 km diameter C asteroid 141 Lumen, the C2 carbonaceous chondrite Murchison, and a carbonaceous construct are shown in Fig. 1.

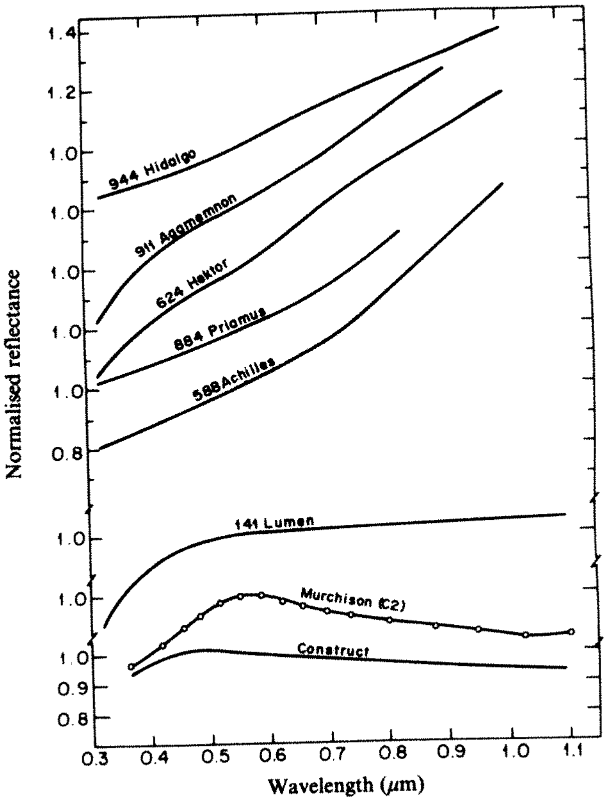

Fig. 1 Reflectance spectra of RD and C asteroids, the C2 carbonaceous chondrite Murchison, and a carbonaceous construct normalised at a wavelength of 0.56 µm. The construct is composed of 85% montmorillonite, 8% magnetite and 7% carbon (charcoal)[5]. The C asteroid is 141 Lumen. The RD asteroids are 588 Achilles, 624 Hektor ($p_v = 0.038$) and 911 Agamemnon ($p_v = 0.035$) in the preceding Trojan cloud, 994 Priamus of the following Trojan cloud and 944 Hidalgo[7]. Hidalgo is a unique asteroid in a comet-like orbit with a semimajor axis of 5.8 AU. Polarimetry and visual photometry suggest that Hidalgo may have a very low albedo[6].

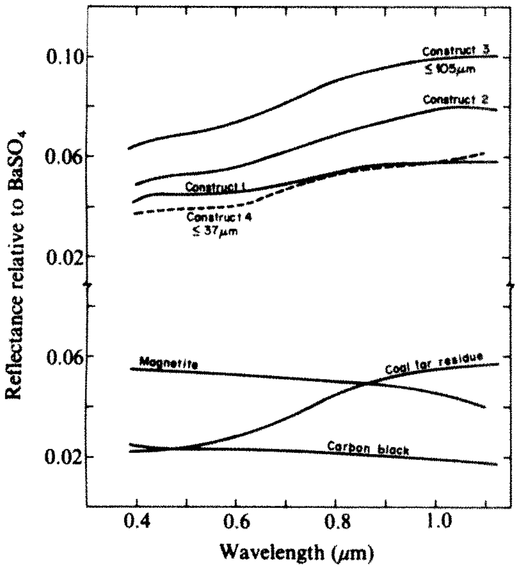

Fig. 2 The reflectances relative to $BaSO_4$ of several opaque substances and three carbonaceous constructs. Construct 1 is composed of 85% montmorillonite, 14.5% coal tar residue and 0.5% carbon black. Construct 2 is composed of 85% montmorillonite and 15% coal tar residue. Constructs 3 and 4 are composed of 85% montmorillonite, 7.5% magnetite and 7.5% coal tar residue. The particle size in constructs 1, 2 and 3 is ≤105 μm but ≤37 μm for construct 4.

However, there are low albedo asteroids which do not fit into the above scheme: some of the Trojan asteroids at two of the lagrangian points of Jupiter, some of the Hilda asteroids near the 2:3 commensurability with Jupiter, and the unusual asteroid 944 Hidalgo which has the second largest semi-major axis (5.8 AU) of any known minor planet. These objects have been labelled RD by Degewij and van Houten[6] as they are very dark ($p_v \sim 0.02$–0.04) and, unlike the neutrally grey C asteroids, are spectrally reddened between 0.4 and 1.1 μm. The normalised reflection spectra of several RD objects reported by Chapman and Gaffey[7] are shown in Fig. 1.

Degewij and van Houten[6] have suggested two possible compositions to explain this unique combination of low albedo and red colour: (1) a material similar to the calcium-rich angrite (visual reflectance ~0.055); and (2) following a suggestion from Gaffey, an iron-rich clay mineral similar to the matrix material of C1 and C2 carbonaceous chondrites but without the usual attendant opaque phases (magnetite and carbon-rich compounds). Both suggestions have difficulties. In the first, the connection with the angrite seems implausible as the single known angrite is classified with the eucrites as basaltic achondrites[8], which are the products of high-temperature processes that are unlikely to have occurred commonly in small bodies in the outer system. It is also difficult to resolve the absence in the RD spectra of the band near 1 μm prominent in the angrite spectrum. The second suggestion is simply incompatible with the extremely low visual geometric albedo of RD objects.

We propose that the nature of the RD objects can be explained better by the presence of very opaque, very red, polymer-type organic compounds, which are structurally similar to aromatic-type kerogen. Kerogen-like substances compose the bulk of the organic material in some carbonaceous chondrites[9]. These compounds probably result from non-biological processes in the early solar nebula—for example, materials from Fischer–Tropsch-type reactions between carbon monoxide and hydrogen at a few hundred degrees kelvin with iron metal, magnetite or silicate clay dust as possible catalysts may have been thermally altered to produce aromatic rich opaques[9–12].

Kerogen substances can be obtained by removing all solubles from ordinary coal tar. The spectral characteristics of the solubles have been investigated elsewhere[13]. The spectrum of the kerogen-like carbonaceous non-soluble residue left after dissolving coal tar in solutions of hexane, toluene and acetone measured with the Cornell goniometer is compared with those of carbon black and magnetite in Fig. 2. Of importance is the extremely low reflectance (0.02) of the coal tar residue in the visible compared to magnetite, and the strong increase in reflectance at the longer wavelengths compared with carbon black. Also shown in Fig. 2 are the spectra of various combinations of powdered montmorillonite, magnetite, the non-soluble residue from coal tar, and carbon black. Constructs 1, 2 and 3 are composed of grains ≤105 μm. Construct 4 is the same composition as construct 3 but composed of grains ≤37 μm. The decrease in reflectance with decreased grain size is opposite that observed for the clay minerals montmorillonite and chlorite[14] but can be explained by the extremely efficient coating ability of finely divided, opaque coal tar residue in a manner similar to that caused by submicrometer particles of lampblack. The normalised spectral reflectance curve of constructs 3 and 4 are compared with the normalised spectra of two RD asteroids in Fig. 3.

All constructs provide good matches to the observed photometric properties of the RD objects: low albedos and very red spectral reflectance curves. It is not necessary to postulate analogues of basaltic achondrites or opaque-poor clays. Mixtures of clays, magnetite, carbon black and an opaque, carbon-rich substance similar to terrestrial kerogen match the observed properties of RD asteroids and are cosmochemically likely to have been produced in the outer Solar System.

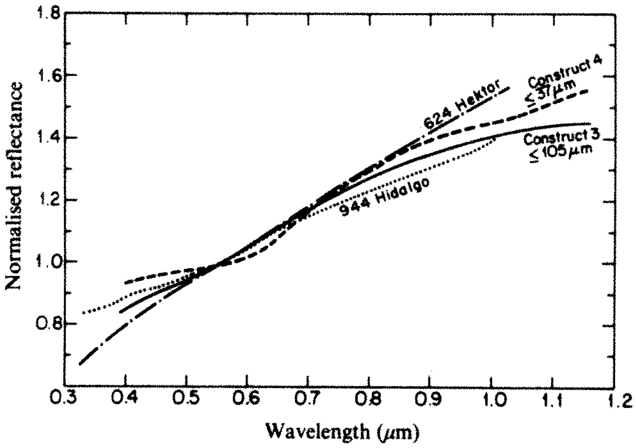

Fig. 3 The normalised reflectance of constructs 3 and 4 compared with two RD asteroids: 944 Hidalgo and 624 Hektor.

Our essential ingredient—the non-soluble residue—may have required lower temperatures for its formation and preservation than the carbonaceous materials in the carbonaceous chondrites and C asteroids. It is significant that a carbon-rich extract from the C3V meteorite Allende measured by Johnson and Fanale[4] has a spectrum unlike that of the non-soluble residue, but similar to that of carbon black, that is, the reflectance increases towards the shorter wavelengths and falls towards the IR. We suspect that the carbonaceous residue from Allende is not representative of the more primitive and unaltered carbon-rich material found in RD objects. This suggestion would explain why RD objects have not been detected closer than ~4 AU from the Sun, and implies that beyond the orbit of Jupiter RD material might replace C asteroid material as the typical condensate. If this suggestion is valid, the rocky material in comets should resemble RD material more closely than that found in the C asteroids.

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IR brightness and eclipse cooling of Saturn's rings

I. G. Nolt*, E. W. Barrett*, J. Caldwell†, F. C. Gillett‡, R. E. Murphy§, J. V. Radostitz|| & A. T. Tokunaga¶

* Atmospheric Physics and Chemistry Laboratory, NOAA, Boulder, Colorado 80303
† Department of Earth and Space Science, SUNY, Stony Brook, New York 11794
‡ Kitt Peak National Observatory, PO Box 26732, Tucson, Arizona 85726
§ Code SL4, NASA Headquarters, Washington, DC 20546
|| Department of Physics, University of Oregon, Eugene, Oregon 97403
¶ Steward Observatory, University of Arizona, Tucson, Arizona 85721

Equatorial scans of Saturn at 20 μm wavelength, obtained with the Mayall 4-m telescope in 1978–79, show a continuing decrease in the specific brightness of the A and B rings as the ring plane projection approaches an edge-on apparition. The decreased brightness of the previously dominant B ring reveals more clearly a large (~30%) difference in brightness between the east and west ansa portions of the C ring, in contrast to a barely discernible difference for the other ring ansae. The amount of eclipse cooling is compatible with a C ring particle size of ~1 cm. We propose here that the B ring brightness variation could partially result from a decrease of absorbed insolation by a modest amount of visible scattering.

Over the past few years we have monitored the 20 μm IR brightness of Saturn's rings with respect to the changing inclination of the ring plane toward the Sun (B'). During this time a large change in ring tilt has occurred. The ring plane attained its maximum tilt of about 26° in 1973 and will be edge-on in early 1980. On four occasions since March 1977 we have measured the ring brightness temperatures with the Kitt Peak National Observatory (KPNO) 4-m Mayall telescope and closed-cycle-cooler photometer system. The nominal 1 mm cold aperture in the focal plane produces a typical beamwidth (FWHM) of 2 arc s.

Figure 1 shows the equatorial scan obtained in January 1979 for a solar elevation angle relative to the ring plane (B') of −6.5°. A computer model fit to the scan is also shown. The model assumes a uniform brightness for each ring component and is more fully described elsewhere[1]. We have normalised the ring brightnesses relative to a disk centre temperature of 89.4±2 K, which assumes that the temperature has decreased by 0.5 K since our first March 1977 observation due to the 2% increase in Saturn's heliocentric distance. This correction assumes equal solar and internal heat sources[1–3].

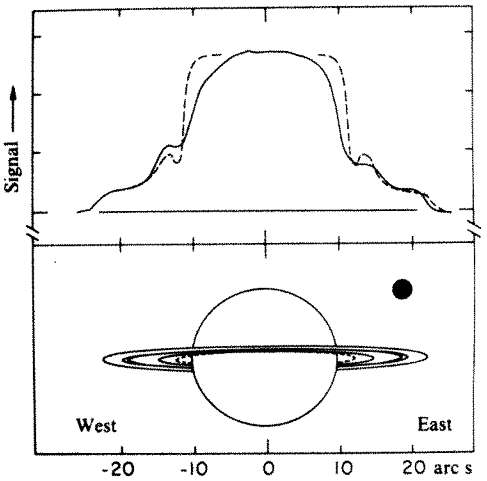

Fig. 1 Equatorial scan of Saturn at 19.7 μm and $B' = -6°.5$ obtained on 10–11 January 1979. The diameter (FWHM) of the telescope point source response function is shown by the cross-hatched circle. The dashed line shows the result of a two-dimensional convolution of the telescope function and the Saturn geometry which defines the ring brightness temperatures. In this calculation a uniform brightness temperature is assumed for each ring component so that the discrepancy between the model result (- - - -) and the experimental scan (——) at the C-ring ansae shows the difference attributed to eclipse cooling.

Comparing these results with our 1978 measurements confirms the pronounced difference in brightness between the east and west ansa positions of the C ring. The fact that a similar difference in brightness was evident in these data (see Fig. 1, ref. 4), when the larger ring inclination of ~12° excluded most of the B ring contribution to the beam at the C ring ansae positions, leads us to associate the observed brightness difference with the C ring. The brightness temperature for the east C ring ansa position is 81.5 K and for the west ansa 85 K, based on the above disk centre temperature of 89.4 K. The post-eclipse east ansa segment is 0.7 as bright as the corresponding west ansa segment.

Aumann and Kieffer[5] calculated particle size versus eclipse heating and cooling for possible ring materials. The observed amount of eclipse cooling, taking into account the ~2,000 s from eclipse exit to the east ansa orbit position, suggests a mean particle size of the order of 1 cm. The A and B rings do not show a significant brightness difference between the east and west ansa segments. Even allowing for the longer orbital periods in this case, the evidently much smaller eclipse cooling at the ansae would seem to exclude the same size composition for these rings. The C ring continues to show no decrease in brightness with respect to changing solar elevation, in contrast to the A and B rings[1].

Table 1 summarises the average ansa brightness temperature of the B ring over the observable range of ring inclination. In Fig. 2 these results are compared with three different ring models with respect to the B' dependence. While the multiple scattering model of Kawata and Irvine[6] is notable for its excellent match to

Table 1 20-μm brightness temperatures for the B-ring adjusted to a common heliocentric distance of 9 AU

Saturnicentric solar latitude $\lvert B'\rvert$	B-ring brightness temperature (K)	Year of observation	Refs
24°.8	90.0±3	1971	8
26°	93.8±2	1972–73	9–11
16°.3	84.3±2	1977	1
11°.8	77.5±3	1978	This work
6°.5	74.0±3	1979	This work

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visible observations, this model is incompatible with the observed B' dependence of the B-ring brightness at 20 μm.

The discovery of dark carbonaceous ring systems around Uranus and Jupiter led us to attempt to fit the B-ring brightness temperature with a simple two-component model consisting of dark particles sandwiched within a haze of small, icy particles. In this model the C ring is composed of dark particles alone, the less dense ice particles having been swept away by the Poynting–Robertson effect. Significant scattering of the visible solar insolation in the A and B ring occurs in the overlying layers of bright icy particles while the principal absorption and IR emission occurs in the embedded dark material. The dark material comes into thermal equilibrium with solar and planetary radiation components. The IR radiation in the direction of the Earth from the ring has three components: (1) the attenuated direct rays from the embedded dark particles; (2) the radiation scattered in the Earth direction by all points of the upper bright particle layer; and (3) the re-emission of radiation absorbed by the bright upper layer (the absorption of solar radiation by the bright layer is considered negligible).

In the calculation illustrated in Fig. 2, the solar constant is 17.2 W m^{-2} at 9 AU. A good fit is obtained for a total optical depth of 1 in the visible with the embedded dark layer having an optical depth of 0.90 and the bright particle layers over and underlying the dark particles an optical depth of 0.05 each. At 20 μm we assumed the same optical thickness for each layer as in the visible, and a single scattering particle albedo of 0.2 for the ice type particles. Finally, we assumed a constant thermal flux from Saturn of 3 W m^{-2} which accounts for the edge-on temperature limit of 70 K for zero inclination. The essential feature of this model is the scattering of visible solar insolation by a small particle fraction (~10%) of the total optical depth. The remaining specifications for a complete model would, of course, have to consider the optical and radar properties.

The experimental data can be equally well described by a $\sin^{1/4}|B'|$ dependence plus a constant planetary heating term equal to about 60 K. This is equivalent to an idealised Lambert surface which may represent the behaviour of a multi-layer particle model with certain simplifying assumptions according to Pollack[7]. Our most recent point for $\sin|B'| \cong 0.1$ suggests a possible inflection in the curve such as is seen in the two-component model result. Observationally it should be possible to obtain measureable ring signals for $\sin|B'|$ as small as 0.05. Such a measurement should be attempted in the latter part of 1980.

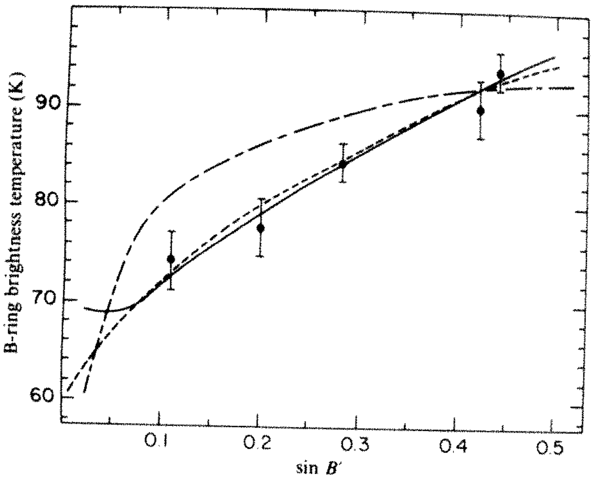

Fig. 2 The B ring brightness temperatures versus the sine of the solar elevation angle. The experimental data are given in Table 1. The three models compared here are: (1) ——, a two-component model discussed in the text; (2) - - - -, a $\sin^{1/4}|B'|$ plus constant dependence equivalent to Lambert type behaviour; and (3) — — — —, the results computed by Kawata and Irvine for a multiple-scattering model (their Model B).

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The photoelectrochemical effect at a p-GaP electrode

J. F. McCann

Physics Department, The University of New South Wales, Kensington, NSW 2033, Australia

L. J. Handley

Chemistry Department, University of Texas at Austin, Austin, Texas 78712

Increasing attention is being focused on the properties and behaviour of p-type semiconductor electrodes. For instance, p-type GaP electrodes have been used in photoelectrochemical cells for water splitting[1], CO_2 reduction[2] and N_2 fixation[3]. We report here the quantum efficiency–potential characteristics for H_2 evolution at an irradiated p-GaP electrode. The curves have been evaluated using a Schottky barrier model of the semiconductor/electrolyte interface[4].

The H_2 evolution reaction at a p-type semiconductor electrode involves the transfer of photogenerated electrons from the conduction band edge (and via surface states) to H_3O^+ acceptor levels. For wide band gap non-corroding p-type semiconductors the H_2 evolution reaction is driven, in certain conditions, by a built-in overpotential (U_c) given by:

$$U_c = E_g - 1.23 - V_{sc}^{s-c} - \Delta E_F \qquad (1)$$

where E_g is the energy gap, V_{sc}^{s-c} is the potential drop across the space charge layer when the p-type semiconductor cathode is short-circuited to the anode and ΔE_F is the difference between the Fermi level and the valence band. An energy diagram of a p-type semiconductor/electrolyte/metal cell is shown in Fig. 1.

Solid state kinetics is favoured over electrochemical kinetics when U_c is large, which is frequently the case for wide band gap semiconductors. For p-GaP it was estimated that $U_c \sim 1.2$V. Therefore, for conditions which include low light intensities, sufficient cathodic bias and concentrated electrolyte it is appropriate to apply Schottky barrier theory to describe the quantum efficiency of H_2 evolution from a p-GaP electrode as a function of potential. According to this theory the quantum efficiency (η) is given by[4]:

$$\eta = 1 - \frac{\exp[-\alpha(\lambda) W_0 \Delta V_{sc}^{\frac{1}{2}}}{1 + \alpha(\lambda) L_n} \qquad (2)$$

where $\alpha(\lambda)$ is the absorption coefficient at a specified wavelength λ, W_0 is the space charge width for 1 V across the depletion layer, L_n is the electron diffusion length and ΔV_{sc} is the potential drop across the semiconductor space charge layer. The parameter ΔV_{sc} is the difference between the potential at the semiconductor surface and the flat band potential. The

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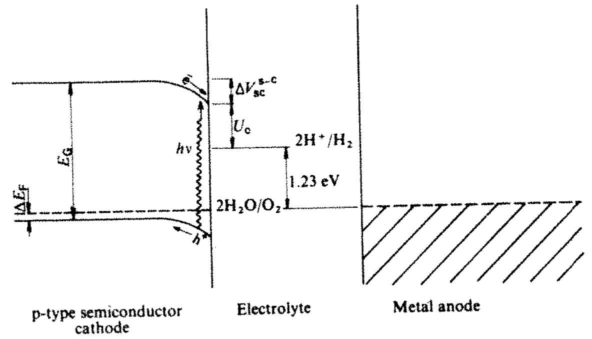

Fig. 1 Energy level diagram of a p-type semiconductor/electrolyte/metal system.

difference is proportional to the applied potential. For equilibrium short-circuit conditions ΔV_{sc} equals ΔV_{sc}^{s-c} in the absence of surface states.

An ohmic contact to a single crystal of p-type GaP (Zn doped; 111 face; $N_A \sim 2 \times 10^{16}\ \text{cm}^{-3}$; $\rho \sim 2\ \Omega\text{cm}$) was formed by soldering an In–Zn alloy onto the back of the sample. The crystal was then fabricated into an electrode by positioning it in a Teflon holder and fixing it with epoxy resin. Before the experiment the p-GaP electrode was etched in a HNO_3 : HCl (2 : 1) mixture for 2 min.

The cell p-GaP/1M NaOH/Pt was studied. Nitrogen was bubbled through the electrolyte for 20 min before the experiment. Monochromatic irradiation was obtained by passing polychromatic light from a 900 W xenon source through a high intensity Bausch and Lomb monochromator. Photocurrent-voltage curves were measured and recorded (potentiostatically) in the usual manner whilst irradiating the p-GaP electrode with light of wavelengths 4,400, 4,500 and 4,600 Å. Irradiation intensities were measured with a Zeiss thermopile model VT Q3/A. The quantum efficiency-voltage relationships were determined from the photocurrent-voltage curves using the expression:

$$\eta = \frac{i_{ph} - i_{dark}}{N_{ph}(O)} \equiv \frac{\text{No. of photoelectrons}}{\text{No. of incident photons}} \tag{3}$$

where i_{ph} and i_{dark} are the electrode current densities with and without illumination respectively.

Quantum efficiency-potential curves were calculated from the photocurrent-voltage curves at three different wavelengths (λ = 4,600, 4,500 and 4,400 Å). The $\alpha(\lambda)$s for GaP at these wavelengths were $< 5 \times 10^4\ \text{cm}^{-1}$. If $\alpha(\lambda) \gtrsim 10^5\ \text{cm}^{-1}$ surface recombination effects which become significant due to the high concentration of hole–electron pairs created close to the surface have to be taken into account in the expression for η.

Table 1 Parameters calculated from the quantum efficiency–voltage curve fitting analysis for irradiated p-GaP in 1M NaOH

Wave-length (Å)	(Photocurrent)^2 –flat band potential (V versus SCE)	$\alpha(\lambda)W_0$	Mean deviation (%)	$\alpha(\lambda)L_n$	Mean deviation (%)
4,400	−0.64	0.92	2.0	2.2×10^{-3}	1.8×10^3
4,500	−0.64	0.64	2.8	9.8×10^{-4}	1.0×10^3
4.600	−0.64	0.27	3.8	3.4×10^{-4}	1.0×10^3

Table 2 Comparison of the $\alpha(\lambda)W_0$ terms from curve fitting with $\alpha(\lambda)W_0$ terms calculated using $\alpha(\lambda)$s from ref. 6

Wavelength (Å)	$\alpha(\lambda)$ (cm^{-1}) (from ref. 6)	$\alpha(\lambda)W_0$* (using $\alpha(\lambda)$ from ref. 6)	$\alpha(\lambda)W_0$ (curve fitted values)
4,400	2.24×10^4	0.55	0.92
4,500	1.57×10^4	0.39	0.64
4,600	6.16×10^3	0.15	0.27

* W_0 was calculated as $2.47 \times 10^{-5}\ \text{cmV}^{-1}$ from equation (4) using $\varepsilon = 11$ (ref. 7).

Table 3 Comparison of the calculated and experimental ratios[6] of the absorption coefficients for p-GaP in 1M NaOH

Ratios	Experimental (from ref. 6)	Curve fitting
$\alpha(4{,}400\ \text{Å})W_0/\alpha(4{,}500\ \text{Å})W_0$	1.4	1.4
$\alpha(4{,}500\ \text{Å})W_0/\alpha(4{,}600\ \text{Å})W_0$	2.5	2.4
$\alpha(4{,}400\ \text{Å})W_0/\alpha(4{,}600\ \text{Å})W_0$	3.6	3.4

A nonlinear least-squares computer program was used to fit equation (2) to the experimental quantum efficiency–voltage curves. Two adjustable parameters, $\alpha(\lambda)W_0$ and $\alpha(\lambda)L_n$, and the flat band potentials determined from (photocurrent)^2–potential plots were used. Table 1 lists the flat band potentials and also the values determined for $\alpha(\lambda)W_0$ and $\alpha(\lambda)L_n$, from the least squares analysis of each curve together with the respective mean deviations of the latter parameters.

The experimental and theoretical quantum efficiency–voltage curves are shown in Fig. 2. The mean deviations of the $\alpha(\lambda)W_0$ terms were small (<4%) whilst those of the $\alpha(\lambda)L_n$ terms were large (~1,000%). The large uncertainties in the $\alpha(\lambda)L_n$ terms are due to the fact that their magnitudes are small in comparison to 1.0 which in turn means that their values cannot be determined accurately via a nonlinear least-squares fit of equation (2). Physically, the latter result implies that the contribution of electrons, photogenerated in the bulk region of the semiconductor, to η, is small.

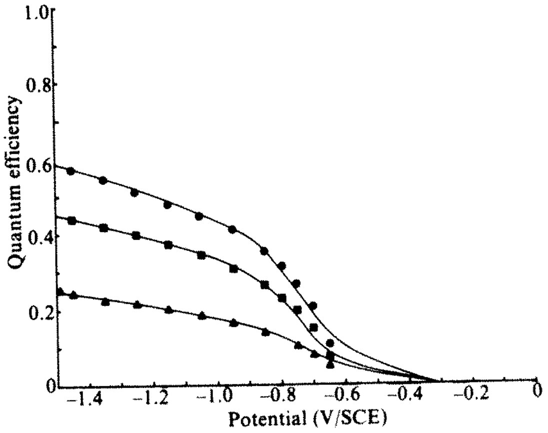

Fig. 2 Experimental (solid lines) and calculated (points) quantum efficiency versus voltage curves for p-GaP in 1M NaOH illuminated by 4,400 (●), 4,500 (■) and 4,600 (▲) Å light.

The parameter W_0 is defined by[4]:

$$W_0 = \left(\frac{2\varepsilon\varepsilon_0}{e_0 N_A}\right)^{1/2} \tag{4}$$

where ε_0 is the permittivity of a vacuum, e_0 is the electronic charge, ε is the semiconductor dielectric constant and N_A is the acceptor density. Table 2 lists the $\alpha(\lambda)W_0$ values obtained from the computer fitting with the $\alpha(\lambda)W_0$ values calculated using the $\alpha(\lambda)$s that have been reported previously for the respective wavelengths[6] and W_0 calculated from equation (4). The agreement between the $\alpha(\lambda)W_0$ terms calculated by the two different methods for each wavelength is satisfactory (within a factor of two) considering the uncertainty in the value of N_A.

A quantitative analysis of the $\alpha(\lambda)W_0$ terms (determined from the least squares fitting) consisted of comparing their ratios with the ratios of the p-GaP absorption coefficients for the respective wavelengths that have been reported previously[6].

The literature and computer fitted ratios are compared in Table 3 and the agreement is close (within 6%). Hence for the first time a quantitative correlation has been observed between the experimental quantum efficiency–potential curves for a p-GaP electrode and the respective fitted curves derived from a Schottky model of the semiconductor/electrolyte interface.

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Analogous Upper Proterozoic apparent polar wander loops

J. D. A. Piper

Department of Geophysics, University of Liverpool, PO Box 147,
Liverpool L69 3BX, UK

The ~1,100 Myr Grenville mobile belt of the Laurentian (North American) Shield yields a record of uplift magnetisations defining a closed apparent polar wander (APW) loop[1–4] nearly identical to the contemporaneous palaeomagnetic records from the Sveconorwegian belt of the Fennoscandian Shield and ~1,100–800 Myr supracrustal successions of the African Shield. The palaeomagnetic data reported here show that these Shields were contiguous at this time[4], and comparison with the pre-1,100 Myr palaeomagnetic record defines a relative rotation of the Fennoscandian Shield with respect to the other major shields at ~1,100 Myr, although they remained in close proximity until late Precambrian times. Tectonic and palaeomagnetic correlations between the major shields suggest that this motion represented an early fragmentation of rigid sialic crust. The restriction of crustal mobility to long linear mobile belts and the progressive contraction of anorogenic magmatism both define a gradual consolidation of the Proterozoic crust as temperature gradients declined.

Considerable attention has recently been devoted to determining the tectonic significance of palaeopoles from the Grenville Province[1–4]; a concerted evaluation has involved reconsideration of the Grenville palaeopoles in the context of the uplift and cooling history of this plutonic terrain[3,5], attempts to define the APW path from the remainder of the Shield during this interval[4,6], and isotopic studies using $^{40}Ar/^{39}Ar$ step heating determinations and diffusion characteristics to calculate both an age and synchronous temperature for minerals from the belt[7]. A relative chronology of the Grenville poles has been established using thermochron zones, which assumes that cooling and magnetisation of the belt followed the scheme defined by K–Ar mica ages; the mean poles defined by selected age intervals are illustrated in Fig. 1*a* (after ref. 2). They define the motion of APW as a closed loop although the age limits of the zones are regarded only as a first approximation to the ages of magnetisation[3,7]. Two more direct estimates by Berger *et al.*[7] use thermochronometry to estimate the ages of TRM components in the Haliburton intrusion (Hb_1 and Hb_3 in Fig. 1*a*) at ~980 and 820 Myr respectively.

The latest assessments agree that the Grenville loop represents an integral part of the total Laurentian APW path[3,4,7] and defines a post-1,030 Myr sequence which fits the sporadic coverage of the same interval from the remainder of the Shield[3,4]. Following refs 2 and 6 the earliest part of the Grenville loop in Fig. 1*a* is connected to a swathe defined by other poles dated 1,100–1,030 Myr. In addition, careful demagnetisation studies[6] have identified a time sequence of palaeopoles from the Jakobsville Formation (J_{1C}–J_{1SA} in Fig. 1*a*) which the authors suggest defines APW motion over the time interval 1,070–1.040 Myr. The youngest part of the Grenville loop also correlates with a range of palaeopoles from elsewhere in the Shield[3,6] although ages of magnetisation are poorly defined. Because most of the poles from the Grenville Province are

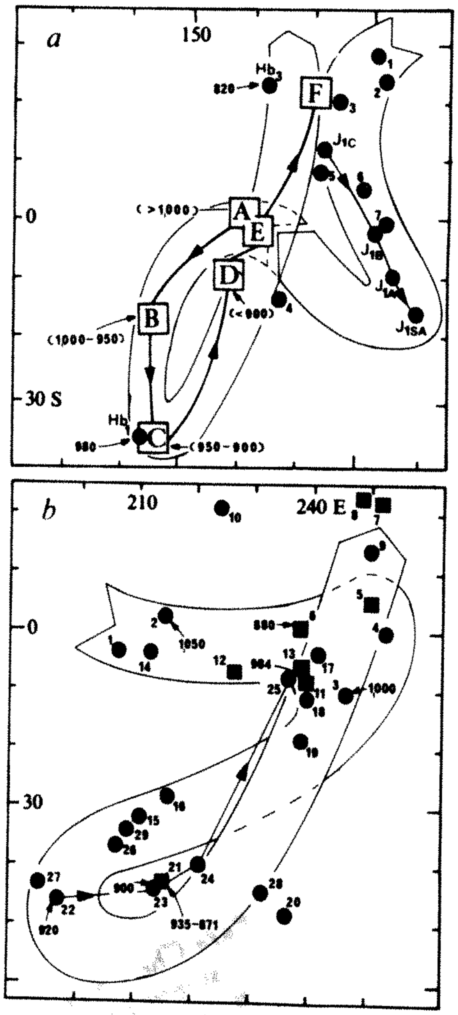

Fig. 1 Laurentian palaeomagnetic poles for the interval ~1,100–800 Myr. Numbered poles with ages in brackets are 1, Mamainse lavas, normal (1,070); 2, Portage Lake lavas (1,100); 3, Copper Harbour lavas (1,080); 4, Nankoweap Formation (1,000); 5, Freda and Nonsuch Sandstone (1,040); 6, Pikes Peak granite (1,030); 7, Cardenas lavas (1,090). JIC to JISA are the sequence of poles defined from the Jakobsville Formation[6]. The palaeomagnetic data from the Grenville Province are illustrated as mean poles A to F from thermochron zones and age assignments are a first order approximation only[2]; specific magnetisations Hb_1 and Hb_3 are dated by isotopic studies[7]. *b*, Fennoscandian 1,050–800 Myr palaeomagnetic poles (summarised in refs 9 and 10) derived from Sveconorwegian uplift magnetisations (●) and igneous intrusions into pre-Sveconorwegian terrain (■). Poles with age assignments in brackets are: 1, Bamble and Köngsberg metamorphic rocks (1,100–950 Myr); 2, Kragerö intrusions (1,050 Myr); 3, 4, Ålgon, Brattön norites; 5–10, Väby, Tärno (880 Myr), Bräkne-Hoby (975–880 Myr) Fäjö, Listed and Bolshavn dykes; 11, Nistrop sill (984 Myr); 12, Årby dyke (995 Myr); 13, Falun dyke (966–914 Myr); 14–18, intrusions in Sveconorwegian margin ('Schistosity') zone; 19, Tuve dyke; 20, SW Sweden amphibolites; 21, Karlshamn dyke (935–871 Myr); 22, Aana-Sira amphibolites; 23, 24, 25, Rogaland igneous complex A, B, C; 26, Egersund anorthosite: 27, 28 SW Norway farsundite; 29, Hunnedalen dykes (900 Myr.).

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derived from multicomponent magnetisations, uncertainties must surround both their precise position and their age[2]; also the link between the Jakobsville and Grenville poles is not, as yet, completely clear[6]. For these reasons it is not certain that the Grenville loop is anticlockwise (see refs 1, 4, 6, 7) as defined by the thermochron zones[2], and it is possible that polar trends over shorter periods were more complex. However, it is clear that the outward and return paths of the loop were very close together, and bearing in mind uncertainties in individual poles of 5–10° and a conventional APW swathe width of 15–20°, these points are not critical to the analysis that follows although they do impose limitations on the definition of the geological model.

The Sveconorwegian mobile belt of southern Norway and south-west Sweden[8] is a zone of medium-high grade plutonic rocks deformed at 1,100–1,000 Myr, and uplifted and cooled over a long interval after this. The palaeomagnetic data from this belt are shown in Fig. 1*b*. Insufficient K–Ar mineral ages are available to attempt a thermochron analysis[3,5] of these data, but we have the advantage of a contemporaneous body of data from dolerites intruded into cold crust of the adjacent (Svecofennian) zone and linked to Rb–Sr determinations[9]. Agreement with uplift magnetisations of the Sveconorwegian belt is close (Fig. 1*b*). The oldest part of the loop is defined by basement rocks of the Bamble and Köngsberg regions[10] with magnetisations linked to Rb–Sr and K–Ar studies[11] indicating an age of 1,050 Myr, and the base of the loop is linked to age dates in the range 935–900 Myr. The return path is defined in part by magnetisations of the Rogaland igneous complex[10,12,13] of which the latest phase is dated 842 Myr (ref. 14) and pole 25 is inferred to be somewhat younger than this[10].

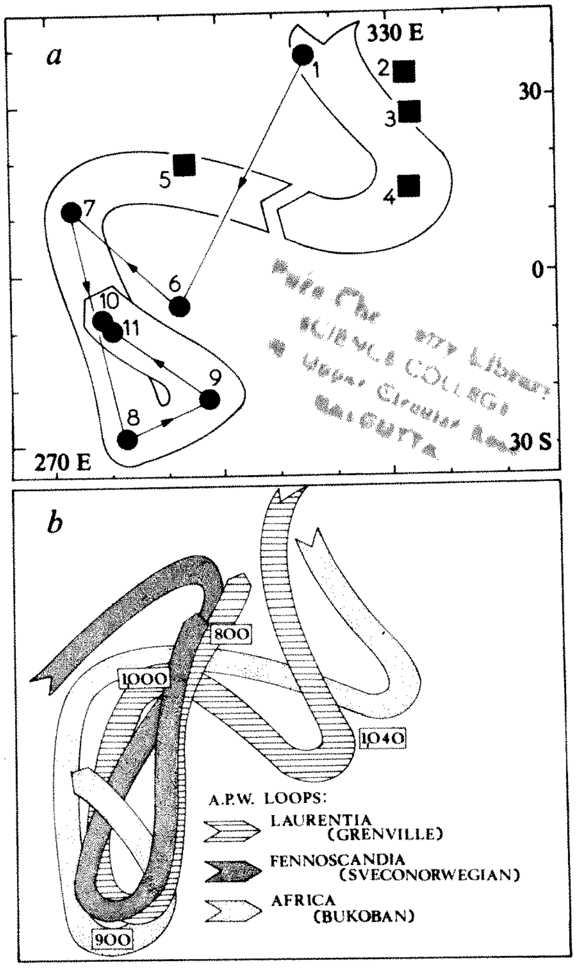

Fig. 2 *a*, African 1,050–800 Myr palaeomagnetic poles[5,15] from margins of the Tanganyika (●) and Kalahari (■) Shields with assigned ages in brackets: 1, Bukoba sandstone (1,000 Myr); 2, Chela sediments; 3, Nosib quartzite; 4, O'okiep intrusives (1,047 Myr); 5, Klein Karas dykes (>890 Myr); 6, Malagarasi sandstone; 7, Kigonero flags (900–1,000 Myr); 8, Gagwe lavas (818 Myr); 9, Manyovu red beds; 10, Moala dolerites; 11, Bukoban dolerites (811 Myr). The dashed line connects the sequence of poles defined from the stratigraphic succession of the Bukoban System; there is overlap between 95% confidence ovals of some of these poles. For pre-1,040 Myr data see ref. 25. *b*, Comparison in size and shape of the three APW loops.

The contemporaneous APW path for Africa is currently less well defined, but is of comparative interest because the data are derived from supracrustal sedimentary/volcanic suites. A loop here is defined by a stratigraphically controlled sequence of poles from the 1,000–800 Myr Bukoban System of Tanzania (ref. 15, Fig. 2*a*) and limited data from southwestern Africa[5,15].

We observe that the 1,050–800 Myr palaeopoles from each of these Precambrian Shields define a flattened APW loop of comparable chronology, size and shape (Fig. 2*b*). The similarity is so close that identical polar movement seems to be recorded relative to each shield; critical points in the correlation are the 1,000–900 Myr and 900–800 Myr links of the Laurentian and Fennoscandian paths and the 1,100–1,040–1,000 Myr arms of the African and Laurentian paths. The principal remaining problem with direct correlation is the distribution of age estimates for the base of the loop ranging from a maximum of 980 Myr from the high blocking temperature component of the Haliburton intrusion[7] to a minimum of 820 Myr for the Gagwe lavas[15]; the latter is a K–Ar determination on lavas exhibiting advanced stages of hydrothermal alteration and is likely to be on the low side.

The tectonic significance of these loops is that when superimposed they yield a reconstruction with the Shields in direct continuity (Fig. 3*b*). The existence of the African–Laurentian link before 1,100 Myr is supported by a range of palaeomagnetic and tectonic evidence[16], and although this link has been disputed[17], I have argued elsewhere that the case rests on an oversimplification of the palaeomagnetic record for the Laurentian Shield[18]. The Laurentian–Fennoscandian configuration however, cannot be reconciled with the pre-1,200 Myr data[16,19,20] which support a contiguous configuration but with the Sveconorwegian and Grenville belts in close alignment (Fig. 3*a*)[20]. This relative motion of the Fennoscandian and African–Laurentian Shields is further indicated by the convergence of their separate APW paths in the correlation of Fig. 2*b*, and the net motion is a clockwise rotation (~110°) of the Fennoscandian Shield about a local axis of rotation in the Baltic Sea.

Whether or not this net rotation was the only motion involved cannot be decided at present because of the paucity of Fennoscandian data relating to the interval of motion (~1,200–1,000 Myr). However, it seems likely that rupture and re-impingement of the two Shields involved some strike-slip motions because there is consistent evidence for dextral motion along the Grenville Front between 1,200 and 1,000 Myr in the form of deformation and displacement of the Labrador Trough[2] and opening of the Keweenawan rift system[21,22]. Impingement of the two shields in a new configuration could account for this large regional stress system, and it also produced crustal thickening of the mobilised continental margins represented by the Grenville and Svecornorwegian belts[2,23]. Direct consequences of this thickening were the extensive intrusion of large anorthosite, gabbro, and related plutons and the protracted crustal uplift[24]; the Grenville Front and 'Schistosity Zone' (the latter separating the Sveconorwegian belt from the previously-stabilised sector of the Fennoscandian Shield) define the abrupt limits of this remobilised terrain. Note also in Fig. 2*b* that there is slight mismatch between the pre- and post-1,000 Myr segments of the African and Laurentian APW curves; this might simply reflect the poor control of the African curve, but may also be interpreted in the context of relative (sinistral) motion between the two Shields at about this time.

Does this fracturing and rotation of the Fennoscandian Shield

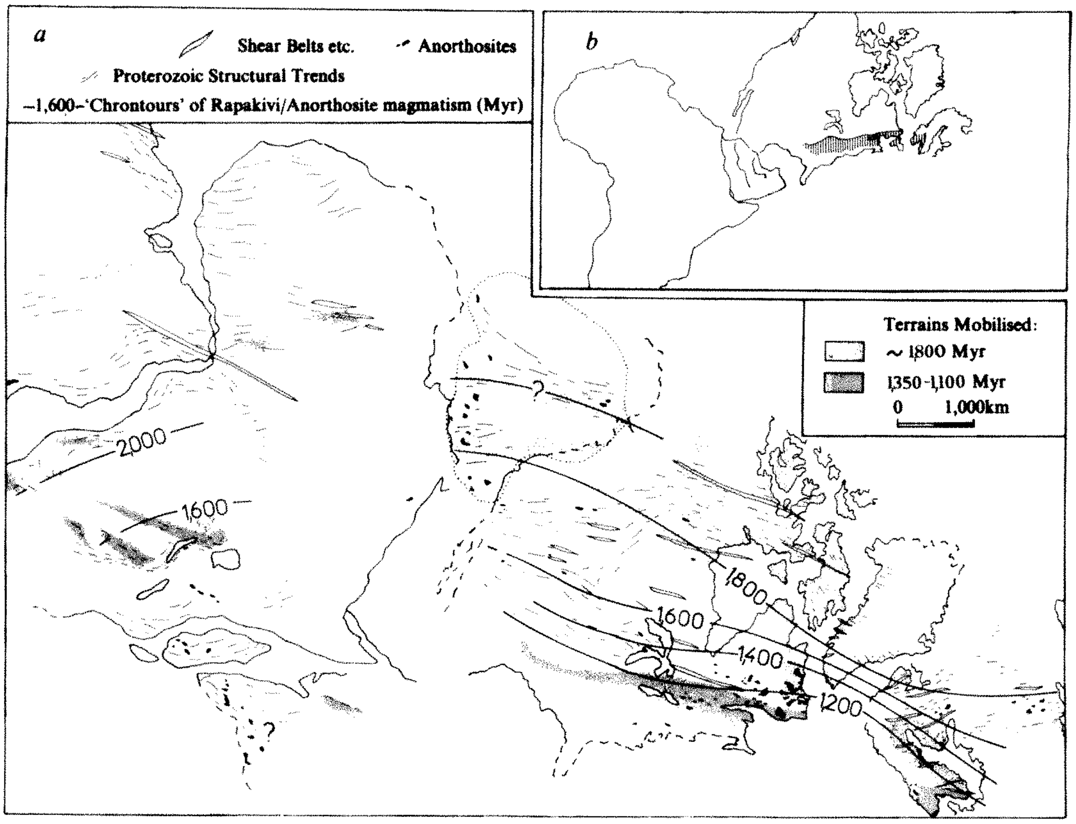

Fig. 3 *a*, The distribution of some of the major shields in Middle Proterozoic times after refs 20, 25, 26 compared with the reconstruction (*b*) derived by superimposing the three 1,050–800 Myr APW loops of Figs 1 and 2. The configuration of the Siberian Shield in *a* is that proposed by Sears and Price[36]; present palaeomagnetic data are not in conflict with this reconstruction[26] but are not yet suitable for a definitive test. The distribution of some major geological features formed between ~2,700 and 1,100 Myr is plotted on *a*, and the chrontours define the approximate time of termination of anorogenic intrusive magmatism belonging to the massive anorthosite/rapakivi granite suite.

at ~1,100 Myr represent the earliest phase in disruption of a consolidating Proterozoic crust? Present palaeomagnetic data bearing on this point are equivocal, although they are consistent with a single configuration of the Fennoscandian and Laurentian Shields to at least 1,700 Myr (refs 25, 26) (and the African and Laurentian Shields to at least 2,100 Myr (ref. 25). There is, however, a strong geological case to suggest that the crustal distribution of Fig. 3*a* represents the approximate form of the continental crust before ~1,100 Myr (the Australian sector of Gondwanaland is omitted from this diagram because relative motions before the Lower Palaeozoic[27] leave uncertainties about its relationship to the other shields). This evidence includes the general alignment of Lower Proterozoic structural trends, mylonite and strike-slip zones[26,26–30] and the pattern of the Rapakivi-massive anorthosite intrusive activity. Conditions appropriate[31] to this anorogenic magmatism, widespread in early Proterozoic times, became progressively more restricted within the zones regenerated during the widespread ~1,800 Myr mobile activity (ref. 32 and Fig. 3*a*) with a gradual restriction to one margin of the Fennoscandian–Laurentian Shield; intrusion ceased altogether at about 1,000 Myr. Figure 3 shows 'chrontours' (based mainly on age data compilations discussed in refs 32 and 26) to describe the contraction of this magmatism across the Proterozoic crust. These appear to reflect declining temperature gradients as this crustal unit cooled so that conditions were no longer appropriate to the high level intrusion of large anorogenic bodies; a comparable trend seems to be present across the African Shield (Fig. 3*a*), although the data points here are too few to be sure of this.

The intrusion of anorogenic bodies persisted longest near the Grenville and Sveconorwegian mobile belts. These and other belts formed during the episode of mobile activity at 1,350–1,100 Myr (ref. 33) tend to form linear and relatively narrow zones separated b sharp structural and/or thermal contacts from adjoining terrains; they are probably more widespread than originally suspected within the African Shield[34]. Their alignment along a great circle trend[16] in the pre-1,100 Myr reconstruction (Fig. 3*a*) began to be dismembered by the post-1,100 Myr motions (Fig. 3b)).

Therefore, the initial break-up of the sialic crust apparently followed closely on the decline of temperature gradients defined by the stage at which anorogenic magmatism could no longer take place outside narrow zones of crustal mobility. This stage is also represented by transcontinental brittle fracturing during the Mackenzie–Sudbury–Gardar–Jotnian igneous episode at ~1,200 Myr (ref. 20). The transition to the scheme of plate tectonics characteristic of Phanerozoic times was, however, a gradual one because ensialic zones of crustal mobility were still developing within the African Shield at 700–500 Myr (ref. 35).

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Oxygen isotopic-hydrographic relationships among recent planktonic Foraminifera from the Indian Ocean

Douglas F. Williams & Nancy Healy-Williams

Department of Geology and Belle W. Baruch Institute for Marine Biology and Coastal Research, University of South Carolina, Columbia, South Carolina 29208

The stable isotopic composition of fossil Foraminifera has become an increasingly important tool in palaeoecological and stratigraphic studies of marine sediments[1-3], prompting several studies of the factors governing the isotopic composition of living and recent Foraminifera. Many studies have documented the good agreement between the inferred depth of growth from oxygen isotopic data[4-8] and the depth stratification found in plankton tows[9-11]. Although results have suggested disequilibrium in some species[12-14], especially among the benthic species[15,16], recent data from Sargasso Sea plankton tows[17-19] and Indian Ocean core top sediments[20,21] suggest that oxygen isotopic equilibrium is nearly achieved in many ecologically important species. In the present study, oxygen isotopic data on as many as eight species and subspecies of planktonic Foraminifera from 52 Indian Ocean core top samples permit a comprehensive comparison of the isotopic–temperature trends across the major oceanographic regions of the Indian Ocean (Table 1). Several shallow-dwelling species (*Globigerinoides ruber*, *G. sacculifer* and *Orbulina universa*) were used as a measure of the upper 50–100 m of the water column (the mixed surface layer). Several deep-dwelling species (*G. truncatulinoides* (left and right coiling varieties), *G. inflata*, *G. scitula* and *G. hirsuta*) were used for comparison with hydrographic data from 200 to 300 m depths. The results show that the isotopic difference between the shallow and deep-dwelling species is directly related to the temperature difference between the surface waters and the depth of the permanent thermocline. This isotopic–temperature relationship suggests that isotopic analyses of species which inhabit different water depths may be used to determine the thermal gradient in the upper water column during the Cenozoic.

The foraminiferal δ^{18}O data are from four sources: widely distributed core tops from Williams[8] and Prell *et al.*[22]; Arabian Sea and Bay of Bengal core tops from Duplessy[23]; and core tops from the southwestern Indian Ocean by Shackleton and Vincent[24]. Temperature and salinity data were obtained for each core top location from the Indian Ocean atlas of Wyrtki[25] and surface temperature maps compiled by the Geophysical Fluid Dynamics Laboratory[26] (Table 1). The salinity–δ^{18}O relationship for Indian Ocean waters was used to estimate the isotopic composition of seawater[27]. The isotopic equilibrium relationship of inorganically precipitated calcite[28] was used to estimate the δ^{18}O of calcite precipitated in isotopic equilibrium at ambient seawater conditions. Linear regression analyses were then used to compare the foraminiferal isotopic data against surface water temperature, and the δ^{18}O of equilibrium calcite at 0, 200 and 300 m.

Strong linear correlations exist between the δ^{18}O of the shallow dwelling species, surface-water temperature and estimated δ^{18}O values for equilibrium calcite at surface conditions (Fig. 1; Table 2). The isotopic composition of the three mixed surface layer species shows depleted values at warmer water temperatures and a steady enrichment at colder temperatures. Individually, the δ^{18}O of *G. ruber* and *O. universa* have the best fit with average surface temperature ($r = -0.89$ and -0.91). Collectively, the δ^{18}O values of all three species have an equally strong correlation with average surface temperature ($r = -0.89$). The slope and intercept of the regression equations are almost identical to those based on equilibrium calcite values and temperature at the same locations (Table 2). The differences in slope and intercept of the regression results for each species (Table 2) reflect the slight differences in calcification temperature due either to seasonal growth or to small depth habitat differences within the mixed surface layer.

Over the same latitudinal and temperature range, however, the isotopic composition of the deep-dwelling *Globorotalia* species shows very little systematic change (Fig. 1). North of the Subtropical Convergence (STC) zone (38°–42° S) the δ^{18}O–temperature gradient for the four *Globorotalia* species together ($r = -0.53$) is 0.05‰ °C^{-1}, <30% of the gradient exhibited in the shallow species and equilibrium calcite. The oxygen isotopic composition of *G. inflata* and *G. truncatulinoides* has a poor correlation with surface temperature ($r = -0.54$ and -0.62). Assuming isotopic equilibrium, calcification among the *Globorotalia* group north of the STC seems to occur at the base of the seasonal thermocline between 200 and 300 m on the basis of estimated δ^{18}O values for equilibrium calcite at these depths (Fig. 1). South of the STC, the δ^{18} of the *Globorotalia* species becomes significantly correlated with surface temperature ($r = -0.89$) with an increase in the δ^{18}-temperature gradient to 0.13‰ °C^{-1} (Fig. 1; Table 2). Individually, *G. truncatulinoides* and *G. inflata* specimens south of the STC exhibit equally strong correlations between their isotopic composition and temperature ($r = -0.93$ and -0.97) (Table 2).

These isotopic trends among the shallow and deep dwelling foraminiferal species have an oceanographic explanation which has important implications for future palaeoceanographic studies. In northern subtropical, equatorial and southern subtropical waters where a steep thermocline exists either year-round or seasonally, the temperature dependent isotopic difference between the deep and shallow dwelling species ($\Delta^{18}\text{O} = \delta^{18}\text{O deep} - \delta^{18}\text{O shallow}$) is large and varies between 3.7 and 1.5‰ (Figs 1 and 2). Δ^{18}O undergoes a systematic decrease toward higher latitudes and decreasing surface water temperature, mirroring the change in the temperature gradient between the surface and depth of the permanent thermocline. At the STC zone, where southern subtropical and northern subantarctic surface water masses converge, Δ^{18}O reaches a minimum of <1‰, corresponding to the region where the thermocline has a minimal thermal gradient. In the subantarctic, Δ^{18}O remains at a minimum, reflecting the lack of a temperature difference between either the season of growth or depth of growth of the deep and shallow species of this region. The same basic Δ^{18}O–temperature relationship exists if the isotopic difference between the *Globorotalia* group and *G. ruber*, or *G. truncatulinoides* and *G. ruber.* is used (Fig. 2).

By calibrating these systematic isotopic differences between pelagic species which inhabit the mixed surface layer and the upper permanent thermocline, the isotopic difference between the average δ^{18}O values of the shallow species and deep *Globorotalia* species could be used to provide an average measure of the temperature difference between the mixed layer and permanent thermocline during different climatic and glaciological states. Likewise, the isotopic difference between a single shallow species (*G. ruber*) and deep species (*G. truncatulinoides*) which have overlapping geographic ranges, could also be utilised. Although all three of the Δ^{18}O–temperature relationships are strongly linear (Table 3), the best fit lines are not parallel but converge towards lower temperatures (Fig. 2). At a temperature maximum of 28 °C, the Δ^{18}O–temperature equations differ by only ±0.25‰ (1 s.d.), which is equivalent to a temperature difference of ±1.2 °C (1 s.d.). An estimated error in the Δ^{18}O (deep–shallow) equation of ±0.33‰ (±1.65 °C) is obtained by comparing observed and estimated Δ^{18}O values. Although more time consuming, the use of an average isotopic

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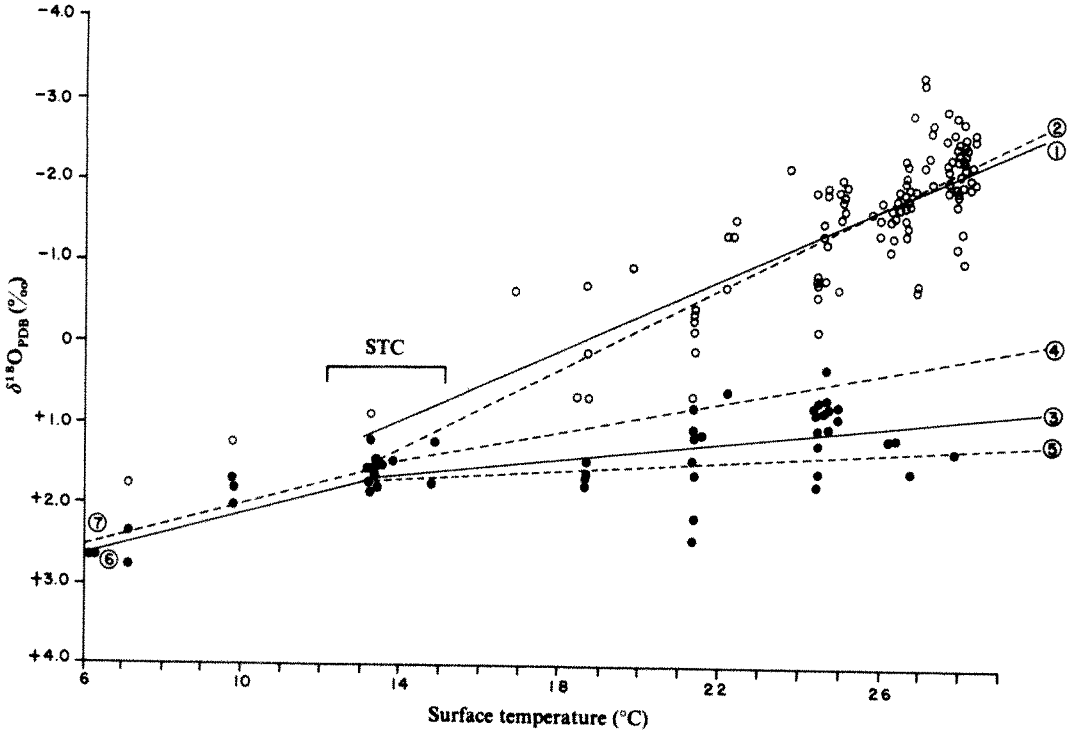

Fig. 1 Linear regression results comparing the $\delta^{18}O$ of the shallow (○) species (*G. ruber, G. sacculifer, O. universa*) and deep (●) species (*G. truncatulinoides, G. inflata, G. hirsuta, G. scitula*) to average surface-water temperature in the Indian Ocean. The temperature range of the subtropical convergence zone (STC) is indicated. The solid lines 1, 3, and 6 represent the best fit lines for all shallow species, all deep species north of the STC, and all deep species south of the STC, respectively (Table 2). The dashed lines represent the best fit lines for equilibrium calcite at 0 m (2), 200 m (4, north of the STC; 7, south of the STC) and 300 m (5, north of the STC) (Table 2). The size fraction of the foraminiferal specimens varied from >63 μm (ref. 24) to >150 μm (refs 8, 22, 23). The isotopic differences between different size fractions of the same species[33,34] probably add to the scatter in the data but do not detract from the results of this study.

Other essential aspects of the analytical techniques were the same in generating the isotopic data: monospecific foraminiferal calcite samples were reacted *in vacuo* in purified phosphoric acid after roasting *in vacuo* for 1 h. The evolved CO_2 gas was purified of water vapour and analysed in a VG Micromass 602 mass spectrometer. All are reported in the conventional δ notation as the ‰ enrichment or depletion in ^{18}O relative to the PDB standard[35]. Detailed analytical procedures can be found in Shackleton and Opdyke[36], Williams *et al.*[37] and Duplessy[23]. Oxygen isotope stratigraphy was used to validate the age of nearly all the core top samples as Recent or late Holocene.

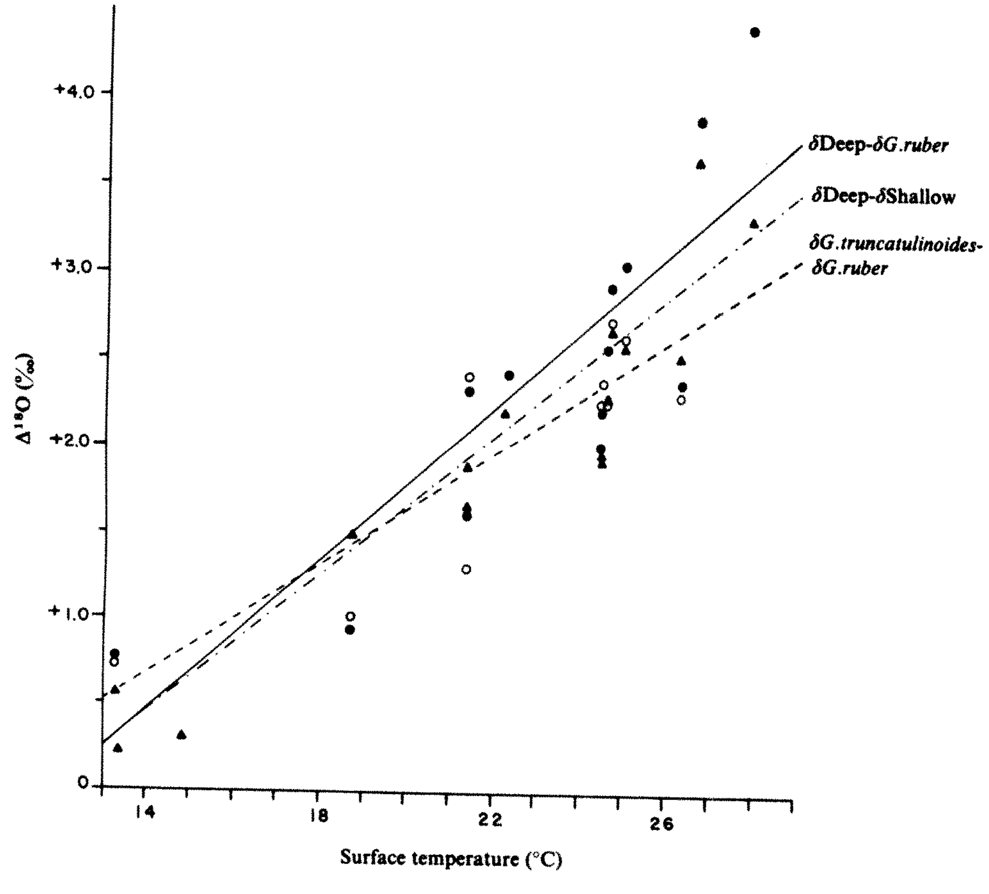

Fig. 2 Linear regression results comparing the oxygen isotopic difference ($\Delta^{18}O$) of average deep – shallow group (▲), average deep group – *G. ruber* (●), and *G. truncatulinoides – G. ruber* (○) with surface water temperature. See Table 3 for exact equations.

Table 1 Foraminiferal, salinity and temperature data

Species	$\delta^{18}O_{PDB}$	$\delta^{13}C_{PDB}$	Core top	Lat	Long	Surface temperature (°C)*	Surface salinity†	$\delta^{18}O_w$‡	Source
Globigerinoides ruber	−1.96	+1.10	366B	23°09′ S	48°08′ E	24.70	35.25	0.40	24
	−1.86	+0.68	363C	23°45′ S	43°10′ E	25.00	35.25	0.40	24
	−1.51	+0.58	361J	25°31′ S	37°45′ E	24.60	35.30	0.40	24
	−1.38	+1.00	389B	30°10′ S	32°04′ E	22.25	35.45	0.49	24
	−0.16	+0.23	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	−1.54	+1.05	V14-101B	08°39′ N	58°34′ E	26.00	35.18	0.42	23
	−1.98	+1.12	V14-104	13°26′ N	53°27′ E	25.15	35.70	0.73	23
	−2.23	+1.24	V14-107	11°51′ N	46°45′ E	26.70	35.95	0.73	23
	−2.57	+0.76	RC9-155	07°24′ N	72°48′ E	28.10	35.00	0.06	23
	−2.33	+1.19	RC12-328	08°57′ N	60°36′ E	27.25	35.15	0.20	23
	−1.44	+0.39	RC12-331	02°30′ N	69°52′ E	28.1	35.10	0.40	23
	−2.47	+0.89	RC17-126	07°00′ N	72°00′ E	27.95	34.50	−0.18	23
	−2.04	+1.20	V19-176	07°07′ N	76°33′ E	27.75	33.85	−0.66	23
	−2.37	+0.99	V19-177	07°35′ N	74°13′ E	27.95	34.25	−0.52	23
	−2.84	+0.89	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	23
	−2.89	+1.16	V19-183	08°07′ N	62°47′ E	26.85	35.30	0.16	23
	−2.31	+0.97	V19-185	06°42′ N	59°20′ E	26.65	35.25	0.20	23
	−1.82	+1.04	V19-188	06°52′ N	60°40′ E	26.65	35.25	0.20	23
	−2.65	+1.12	RC12-339	09°08′ N	90°02′ E	27.95	33.80	−0.37	23
	−2.93	+0.95	RC12-340	12°42′ N	90°01′ E	27.70	33.35	−0.66	23
	−2.77	+1.11	RC12-341	13°03′ N	80°35′ E	27.35	33.50	−0.66	23
	−3.36	+0.92	RC12-344	12°46′ N	96°04′ E	27.10	32.05	−0.34	23
	−2.78	+0.90	RC12-347	09°20′ N	93°20′ E	28.10	33.50	−0.66	23
	−2.66	+0.92	RC14-36	0°28′ S	90°00′ E	28.35	34.23	−0.08	23
	−2.42	+1.08	RC14-39	05°51′ N	90°31′ E	27.95	33.85	−0.42	23
	−2.55	+1.35	RC14-44	05°58′ S	102°27′ E	27.65	32.00	−1.14	23
	−2.45	+0.41	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	8
	−1.82	+0.86	V19-185	06°42′ N	59°20′ E	26.65	35.25	0.20	8
	−1.17	+0.61	V19-202	06°59′ S	41°11′ E	26.35	35.20	0.35	8
	−2.03	+0.78	RC11-147	19°04′ S	112°45′ E	25.05	34.95	0.30	8
	−0.79	+0.02	V20-170	21°48′ S	69°14′ E	24.50	34.85	0.06	8
	−0.86	+0.14	V20-175	22°18′ S	68°00′ E	24.50	34.85	0.06	8
	−0.27	+0.00	V18-207	25°38′ S	87°07′ E	21.40	34.95	−0.04	8
	+0.68	+0.36	RC11-126	30°04′ S	94°25′ E	18.75	35.85	0.73	8
	+0.90	+0.49	E48-22A	39°54′ S	85°25′ E	13.3	35.08	0.32	8
	−1.90	+0.42	RC12-339	09°08′ N	90°02′ E	27.85	34.13	−0.06	8
	+0.67	—	E48-11	29°40′ S	97°32′ E	18.45	35.90	0.73	22
	−1.67	—	V19-202	06°41′ S	41°11′ E	26.35	35.20	0.35	22
	−1.58	—	V19-202	06°41′ S	41°11′ E	26.35	35.20	0.35	22
Globigerinoides sacculifer	−1.86	+1.29	366B	23°09′ S	48°08′ E	24.7	35.25	0.40	24
	−1.58	+1.75	363C	23°45′ S	43°10′ E	25.0	35.25	0.40	24
	−1.37	+1.60	361J	25°31′ S	37°45′ E	24.6	35.30	0.40	24
	−1.37	+2.02	389B	30°10′ S	32°04′ E	22.25	35.45	0.49	24
	−0.38	+1.30	379B	32°23′ S	42°56′ E	21.4	35.50	0.54	24
	−1.37	+1.77	V14-101B	08′39′ N	58°34′ E	26.0	35.18	0.42	23
	−1.69	+2.08	V14-104	13°26′ N	53°27′ E	25.15	35.70	0.73	23
	−1.66	+1.30	DAL7-60	23°15′ N	61°28′ E	25.80	36.45	1.12	23
	−1.57	+1.70	V14-107	11°51′ N	46°45′ E	26.7	35.95	0.73	23
	−2.20	+1.57	RC9-155	07°24′ N	72°48′ E	28.1	35.00	0.06	23
	−2.02	+1.83	RC12-328	03°57′ N	60°36′ E	27.25	35.15	0.20	23
	−1.04	+1.46	RC12-331	02°30′ N	69°52′ E	28.1	35.10	0.40	23
	−1.75	+1.78	RC17-126	7°00′ N	72°00′ E	27.95	34.50	−0.18	23
	−2.34	+1.50	V19-176	7°07′ N	76°33′ E	27.75	33.85	−0.66	23
	−2.13	+1.47	V19-177	07°35′ N	74°13′ E	27.95	34.25	−0.52	23
	−1.95	+1.79	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	23
	−1.94	+1.55	V19-183	08°07′ N	62°47′ E	26.85	35.30	0.16	23
	−2.03	+1.67	V19-185	06°42′ N	59°20′ E	26.65	35.25	0.20	23
	−1.74	+1.70	V19-188	06°52′ N	60°40′ E	26.65	35.25	0.20	23
	−2.31	+1.53	RC12-339	09°08′ N	90°02′ E	27.95	33.80	−0.37	23
	−2.18	+1.55	RC12-340	12°42′ N	90°01′ E	27.7	33.35	−0.66	23
	−2.68	+1.65	RC12-341	13°03′ N	80°35′ E	27.35	33.50	−0.66	23
	−3.26	+1.59	RC12-344	12°46′ N	96°04′ E	27.1	32.05	−1.34	23
	−2.27	+1.51	RC12-347	09°20′ N	93°20′ E	28.1	33.50	−0.66	23
	−2.24	+1.60	RC14-36	0°28′ S	90°00′ E	28.35	34.23	−0.08	23
	−2.45	+1.52	RC14-39	05°51′ N	90°31′ E	27.95	33.85	−0.42	23
	−2.26	+1.87	RC14-44	05°58′ S	102°27′ E	27.65	32.0	−1.14	23
	−1.88	+1.44	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	8
	−1.73	+1.64	V19-185	06°42′ N	59°20′ E	27.65	35.25	0.20	8
	−1.54	+1.97	V19-202	06°59′ S	41°11′ E	26.35	35.20	0.35	8
	−1.86	+1.99	RC11-147	19°04′ S	112°45′ E	25.05	34.95	0.30	8
	−0.73	+1.32	V20-170	21°48′ S	69°14′ E	24.5	34.85	0.06	8
	−0.77	+1.28	V20-175	22°18′ S	68°00′ E	24.5	34.85	0.06	8
	−0.41	+1.12	V18-207	25°38′ S	87°07′ E	21.4	34.95	−0.04	8
	−0.71	+1.13	RC11-126	30°04′ S	94°25′ E	18.75	35.85	0.73	8
	−1.97	+1.97	RC12-339	09°08′ N	90°02′ E	27.85	34.13	−0.37	8
	−1.75	—	V14-101B	08°39′ N	58°34′ E	26.00	35.35	0.42	22
	−2.22	—	RC14-29	10°55′ S	88°19′ E	27.10	34.20	0.01	22
	−2.01	—	RC14-35	0°50′ S	89°57′ E	28.35	34.18	−0.13	22
	−2.56	—	RC14-35	0°50′ S	89°57′ E	28.35	34.18	−0.13	22
	−2.09	—	RC14-37	01°28′ N	90°10′ E	28.25	34.15	−0.18	22
	−1.98	—	RC14-37	01°28′ N	90°10′ E	28.25	34.15	−0.18	22
	−1.88	—	RC11-147	19°04′ S	112°45′ E	24.46	34.95	0.30	22
	−1.56	—	V19-204	10°14′ S	43°49′ E	26.65	35.15	0.30	22

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* See ref. 26. † See ref. 25.
‡ Oxygen isotopic composition of seawater using the salinity/$\delta^{18}O$ relationship for the Indian Ocean[27].

Table 1—*continued*

Species	$\delta^{18}O_{PDB}$	$\delta^{13}C_{PDB}$	Core top	Lat	Long	Surface temperature (°C)*	Surface salinity†	$\delta^{18}O_w$‡	Source
Globigerinoides sacculifer (*continued*)	−1.37	—	V19-204	10°14′ S	43°49′ E	26.65	35.15	0.30	22
	−0.69	—	V29-48	06°16′ S	63°26′ E	26.95	34.80	0.20	22
	−0.71	—	V29-48	06°16′ S	63°26′ E	26.95	34.80	0.20	22
	−1.95	—	V19-185	06°42′ S	59°20′ E	26.65	35.25	0.20	22
	−2.04	—	V19-185	06°42′ S	59°20′ E	26.65	35.25	0.20	22
	−1.55	—	RC17-69	31°30′ S	32°36′ E	22.40	35.40	0.49	22
	−2.30	—	V29-30	03°05′ N	76°15′ E	28.10	35.00	0.30	22
	−1.97	—	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	22
	−2.20	—	RC9-161	19°34′ N	59°36′ E	23.75	36.15	0.97	22
	−1.91	—	RC17-98	13°13′ S	65°37′ E	26.55	34.65	0.11	22
	−1.72	—	RC17-98	13°13′ S	65°37′ E	26.55	34.65	0.11	22
	−0.65	—	RC11-86	35°47′ S	18°27′ E	16.95	35.00	0.30	22
	−0.95	—	RC9-150	31°17′ S	114°33′ E	19.85	35.85	0.69	22
	−2.44	—	V29-29	05°07′ N	77°35′ E	28.00	34.70	0.06	22
Orbulina universa	−1.24	+1.88	366B	23°09′ S	48°08′ E	24.70	35.15	0.40	24
	−0.71	+1.91	363C	23°45′ S	43°10′ E	25.00	35.15	0.40	24
	−0.80	+1.73	361J	25°31′ S	37°45′ E	24.60	35.20	0.40	24
	−0.70	+1.56	389B	30°10′ S	32°04′ E	22.25	35.45	0.49	24
	+0.64	+1.36	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	−1.22	+0.55	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	8
	−1.91	+2.06	V19-185	06°42′ N	59°20′ E	26.65	35.25	0.20	8
	−1.34	+1.68	V19-202	06°59′ N	41°11′ E	26.35	35.20	0.35	8
	−1.79	+1.76	RC11-147	19°04′ S	112°45′ E	25.05	34.95	0.30	8
	−0.59	+1.75	V20-170	21°48′ S	69°14′ E	24.50	34.85	0.06	8
	−0.18	+1.66	V20-175	22°18′ S	68°00′ E	24.50	34.85	0.06	8
	+0.06	+1.73	V18-207	25°38′ S	87°07′ E	21.40	34.95	−0.04	8
	+0.11	+1.84	RC11-126	34°04′ S	94°25′ E	18.75	35.85	0.73	8
	+1.22	+1.60	E48-27A	38°32′ S	79°54′ E	14.85	35.30	0.49	8
	+1.41	+1.73	E48-23A	39°31′ S	83°43′ E	13.40	35.10	0.35	8
	+1.16	+1.60	E48-22A	39°54′ S	85°25′ E	13.30	35.08	0.32	8
	+1.24	+1.20	RC11-120	43°31′ S	79°52′ E	9.80	34.75	0.06	8
	+1.63	+1.73	E45-73A	47°33′ S	114°26′ E	7.20	34.50	0.06	8
Globorotalia scitula	+0.30	—	366B	23°09′ S	48°08′ E	24.70	35.25	0.04	24
	+1.06	−1.16	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	+1.32	−0.17	V19-178	08°07′ N	73°15′ E	27.95	34.70	−0.23	8
	+1.58	+0.72	V19-185	06°42′ N	59°20′ E	26.80	35.25	0.20	8
	+1.19	−0.15	V19-202	06°59′ S	41°11′ E	26.35	35.25	0.35	8
	+1.80	−0.46	RC11-126	30°04′ S	94°25′ E	18.75	35.85	0.73	8
	+1.77	−0.22	E48-22A	39°54′ S	85°25′ E	13.30	35.08	0.32	8
	+1.74	−0.04	RC11-120	43°31′ S	79°52′ E	9.80	34.75	0.06	8
Globorotalia truncatulinoides (L)	+0.72	+1.26	366B	23°09′ S	48°08′ E	24.70	35.25	0.40	24
	+0.77	+1.36	363C	23°45′ S	43°10′ E	25.00	35.25	0.40	24
	+0.73	+1.41	361J	25°31′ S	37°04′ E	24.60	35.30	0.40	24
	+0.81	+0.97	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	+0.84	+1.22	V20-170	21°48′ S	69°14′ E	24.50	34.85	0.06	8
	+1.05	+1.35	V20-175	22°18′ S	68°00′ E	24.50	34.85	0.06	8
	+1.65	+0.88	RC11-126	30°04′ S	94°25′ E	18.75	35.85	0.73	8
	+1.47	+0.47	E48-27A	38°32′ S	79°54′ E	14.85	35.30	0.49	8
	+1.69	+0.34	E48-23A	39°31′ S	83°43′ E	13.40	35.10	0.35	8
	+1.61	+0.43	E48-22A	39°54′ S	85°25′ E	13.30	35.08	0.32	8
	+1.78	+0.78	RC11-120	43°31′ S	79°52′ E	9.80	34.75	0.06	8
	+2.40	+1.13	E45-73A	47°33′ S	114°26′ E	7.20	34.50	0.06	8
	+2.69	+1.14	RC8-63	51°05′ S	129°58′ E	6.10	34.00	−0.18	8
	+1.87	—	E48-22A	39°53′ S	85°25′ E	13.30	35.08	0.32	22
	+1.61	—	E48-22A	39°53′ S	85°25′ E	13.30	35.08	0.32	22
Globorotalia truncatulinoides (R)	+0.78	+1.07	366B	23°09′ S	48°08′ E	24.70	35.25	0.04	24
	+0.75	+1.06	361J	25°31′ S	37°04′ E	24.60	35.30	0.04	24
	+1.12	+0.86	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	+1.12	+0.61	V19-202	06°59′ S	41°11′ E	26.35	35.20	0.35	8
	+1.58	+1.10	V20-170	21°48′ S	69°14′ E	24.50	34.85	0.06	8
	+1.72	+1.39	V20-175	22°18′ S	68°00′ E	24.50	34.85	0.06	8
	+2.15	+1.37	V18-207	25°38′ S	87°07′ E	21.40	34.95	−0.04	8
	+1.71	+0.92	RC11-126	30°04′ S	94°25′ E	18.75	34.85	0.73	8
Globorotalia hirsuta	+1.45	+0.68	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	+2.44	+1.11	V18-207	25°38′ S	87°07′ E	21.40	34.95	−0.04	8
	+1.79	+0.88	E48-27A	38°32′ S	79°54′ E	14.85	35.30	0.49	8
	+1.58	+0.96	E48-23A	39°31′ S	83°43′ E	13.40	35.10	0.35	8
	+1.84	+1.41	E48-22A	39°54′ S	85°25′ E	13.30	35.08	0.32	8
Globorotalia inflata	+1.04	+0.62	366B	23°09′ S	48°08′ E	24.70	35.25	0.40	24
	+0.92	+0.91	363C	23°45′ S	43°10′ E	25.00	35.25	0.40	24
	+0.74	+0.78	361J	25°31′ S	37°04′ E	24.60	35.30	0.40	24
	+0.61	+0.48	389B	30°10′ S	32°04′ E	22.25	35.45	0.49	24
	+1.16	+0.24	379B	32°23′ S	42°56′ E	21.40	35.50	0.54	24
	+1.26	+0.47	V20-175	22°18′ S	68°00′ E	24.50	34.85	0.06	8
	+1.65	+0.74	V18-207	25°38′ S	87°07′ E	21.40	34.95	−0.04	8
	+1.46	+0.59	RC11-126	30°04′ S	94°25′ E	18.75	35.85	0.73	8
	+1.31	+0.60	E48-27A	38°32′ S	79°54′ E	14.85	35.30	0.49	8
	+1.55	+0.38	E48-23A	39°31′ S	83°43′ E	13.40	35.10	0.35	8
	+1.25	+0.62	E48-22A	39°54′ S	85°25′ E	13.30	35.08	0.32	8
	+2.06	+1.15	RC11-120	43°31′ S	79°52′ E	9.80	35.75	0.06	8
	+2.84	+1.21	E45-73A	47°33′ S	114°26′ E	7.20	34.50	0.06	8
	+2.69	+1.14	RC8-63	51°05′ S	129°58′ E	6.10	34.00	−0.18	8

composition for the deep and shallow group would have the advantage of minimising preferential biasing of the isotopic record by bioturbation effects on a single species which undergoes changing relative abundances through short-term climatic cycles[29].

In addition, the results of this study show that the change in the latitudinal $\Delta^{18}O$–temperature gradient in *G. truncatulinoides* and *G. inflata* north and south of the STC can be used to detect changes in the past positions of water mass boundaries such as the STC (the $\delta^{18}O$ of *G. truncatulinoides* changes from 0.06 to 0.12‰ °C^{-1} and the $\delta^{18}O$ of *G. inflata* changes from 0.04 to 0.19‰ °C^{-1} north and south of the STC, respectively). In fossil assemblages where *G. truncatulinoides* is rare or absent, other abundant deep dwelling species such as *G. inflata* may be substituted. South of the STC, the $\delta^{18}O$–temperature best fit line for all the deep dwelling species parallels the best fit lines for equilibrium calcite at both the surface and 200 m.

The $\Delta^{18}O$ trends in the Indian Ocean surface sediments strongly suggest that seasonality may also be recorded in the isotopic composition of fossil assemblages. Results of an isotopic study of living *G. ruber* and *G. truncatulinoides* collected seasonally in the Sargasso Sea[17] have shown that the observed isotopic difference ($\Delta^{18}O$) between *G. ruber* during the summer temperature maximum and *G. truncatulinoides* during the winter temperature minimum ($\Delta^{18}O = 2.0‰$) agrees remarkably well with the estimated $\Delta^{18}O$ of 2.18‰. This estimated $\Delta^{18}O$ value was obtained by extrapolating the average surface temperature at the Sargasso Sea location (23.4 °C) to the $\Delta^{18}O$–temperature relationship for *G. truncatulinoides* and *G. ruber* in the Indian Ocean (Fig. 2). These results suggest that the isotopic composition of certain planktonic foraminiferal species is both a function of depth habitat (particularly in equatorial regions where little seasonal change in the thermocline occurs) and seasonal occurrence.

Of course, to use the modern interglacial $\Delta^{18}O$ relationship to estimate absolute sea-surface temperatures during the Pleistocene, it must be assumed that the deep dwelling species maintain approximately the same mean calcification temperature during glacial times. The near constant isotopic composition of *Globorotalia* species from such widely different oceanographic regions as the Arabian Sea to the Subtropical Convergence suggests that this assumption may be reasonable. It has already been shown that surface isotherms during the last glacial maximum can be reconstructed using the isotopic composition of *G. sacculifer* at equivalent levels in piston cores[30]. It may therefore be possible to map the geographic distribution of $\Delta^{18}O$ at different times to determine the changes in the thermal gradient of the upper water column and to provide a comparison with sea-surface temperature estimates based on micropalaeontological transfer functions[31,32]. In addition, the correspondence between the $\Delta^{18}O$ trend in surface sediments of the Indian Ocean and the seasonal temperature–isotopic relationship in the Sargasso Sea suggests that the seasonality of a region may be decipherable from isotopic studies of multiple species from fossil assemblages.

Table 2 Linear regression analyses of foraminiferal isotopic composition versus water temperature

$\delta^{18}O$*	Surface temperature†	Correlation coefficient	Least squares equations
G. ruber	0 m	−0.89	$\delta^{18}O = 5.48 - 0.28T$
G. ruber	Aug., 0 m	−0.91	$\delta^{18}O = 3.80 - 0.22T$
G. ruber	Feb., 0 m	−0.77	$\delta^{18}O = 6.58 - 0.32T$
G. sacculifer	0 m	−0.66	$\delta^{18}O = 2.36 - 0.16T$
O. universa	0 m	−0.91	$\delta^{18}O = 3.30 - 0.17T$
Shallow‡	0 m	−0.86	$\delta^{18}O = 4.03 - 0.22T$
Deep§, N of STC	0 m	−0.53	$\delta^{18}O = 2.35 - 0.05T$
Deep§, S of STC	0 m	−0.89	$\delta^{18}O = 3.42 - 0.13T$
G. trunc., N of STC	0 m	−0.62	$\delta^{18}O = 2.55 - 0.06T$
G. trunc., S of STC	0 m	−0.93	$\delta^{18}O = 3.28 - 0.12T$
G. inflata, N of STC	0 m	−0.54	$\delta^{18}O = 1.96 - 0.04T$
G. inflata, S of STC	0 m	−0.97	$\delta^{18}O = 3.95 - 0.19T$
equil‖, 0 m	0 m	−0.94	$\sigma^{18}O = 4.81 - 0.25T$
equil‖, 200 m, N of STC	0 m	−0.86	$\sigma^{18}O = 2.71 - 0.09T$
equil‖, 200 m, S of STC	0 m	−0.95	$\delta^{18}O = 3.28 - 0.13T$
equil‖, 300 m, N of STC	0 m	−0.45	$\delta^{18}O = 2.14 - 0.03T$

* Dependent variable—oxygen isotopic composition relative of PDB.

† Independent variable—average temperature at particular depths or seasons.

‡ Average $\delta^{18}O$ of shallow group (*G. ruber* + *G. sacculifer* + *O. universa*).

§ Average $\delta^{18}O$ of deep group (*G. truncatulinoides* + *G. inflata* + *G. scitula* + *G. hirsuta*).

‖ $\delta^{18}O$ of equilibrium calcite at given depths or locations.

Table 3 Linear regression analyses of foraminiferal isotopic differences and water temperature

$\delta^{18}O$	Temperature	Correlation coefficient	Least squares equation
Deep – shallow*	0 m	0.94	$\Delta^{18}O = -2.35 + 0.20T$
Deep – shallow*	ΔT†, 0–200 m	0.88	$\Delta^{18}O = 0.21 + 0.25\Delta T$
Deep – *G. ruber*‡	0 *m*	0.85	$\Delta^{18}O = -2.62 + 0.22T$
Deep – *G. ruber*‡	ΔT‡, 0–200 *m*	0.82	$\Delta^{18}O = 0.49 + 0.25\Delta T$
G. trunc. – *G. ruber*§	0 m	0.87	$\Delta^{18}O = -1.56 + 0.16T$
G. trunc. – *G. ruber*§	ΔT†, 0–200 m	0.70	$\Delta^{18}O = 0.73 + 0.19\Delta T$

* Deep – shallow = ($\delta^{18}O$ of *G. truncatulinoides* + *G. inflata* + *G. scitula* + *G. hirsuta*) – ($\delta^{18}O$ of *G. ruber* + *G. sacculifer* + *O. universa*).

† $\Delta T = T_{0\,\mathrm{m}} - T_{200\,\mathrm{m}}$.

‡ Deep – *G. ruber* = $\delta^{18}O$ of deep group – $\delta^{18}O$ of *G. ruber*.

§ $\delta^{18}O$ of *G. truncatulinoides* – $\delta^{18}O$ of *G. ruber*.

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Test ultrastructure of fusulinid Foraminifera

Harry W. Green II, Jere H. Lipps & William J. Showers*

Department of Geology, University of California, Davis,
California 95616

Foraminifera are separated systematically at the subordinal and suprafamilial level on the basis of their wall ultrastructure[1], yet those elements of the walls are near or at the resolution of the optical microscope[2]. To delineate those elements we have initiated a comprehensive study of the test ultrastructure of calcareous Foraminifera, utilising high voltage transmission electron microscopy (HVTEM) of specimens thinned by ion bombardment[3,4]. We find certain ultrastructural characteristics to be common both to all Foraminifera studied and to all other comparison biological carbonate: for example, organic inclusions are found within and on the boundaries between the crystals of miliolid, fusulinid and rotaliid foraminiferal tests, bivalve shells, coral skeletons and human teeth. Other characteristics are typical of a particular host mineral, but independent of phyletic affinities: for example, the size, shape and abundance of inclusions are different in aragonite crystals from calcite crystals. Still other characteristics are diagnostic of certain groups: for example, crystal sizes and shapes are definitely different for the various suborders of Foraminifera. These ultrastructural characteristics should lead to a better understanding of the relationships between groups of Foraminifera, and to a better understanding of the biological calcification process in general. But more immediately, they provide powerful criteria for the evaluation of ultrastructural modification during and after fossilisation, especially for extinct forms such as the fusulinid Foraminifera.

Fusulinids possess one of the six major wall structure types known in calcareous Foraminifera[1,2]. Termed microgranular, it is characteristic of all species assigned to the extinct Palaeozoic suborder Fusulinina[1]. The microgranules are a few micrometres across, equidimensional and subangular[2]. They were thought to be either agglutinated particles picked up by the organism, or as most workers now believe, to be originally secreted[2]. As recrystallisation is common in fossils of this age, the nature of the microgranules has remained poorly understood.

These microgranules may be aligned with each other in varying degrees, thus forming fibrous or pseudofibrous layers[1]. The layering arrangements in various groups of fusulinids have been well described and are used as systematic criteria[1]. We are not concerned with these structures which are commonly referred to as test wall structure; we studied the internal structure of the microgranules themselves, and the relationship between them.

We consider here the ultrastructure of some well preserved specimens of *Triticites moorei* from the Wayland Shale Member of the Graham Formation (Pennsylvania) 10 miles north-east of Breckenridge, Texas (this is locality C-16 of Cushman and Waters[5]; the original description of *T. moorei* was by Dunbar and Condra[6] from the same formation north-west of Breckenridge). From examination of numerous specimens by optical and scanning electron microscopy, we selected four for detailed study. Examination by HVTEM indicates that the specimens retain their distinctive biological ultrastructure, including preservation of minute organic inclusions.

The degree of preservation of surface morphology is excellent. In some specimens we find evidence of surface damage, perhaps due to current action before incorporation into the sediment, but no evidence of solution effects has been observed.

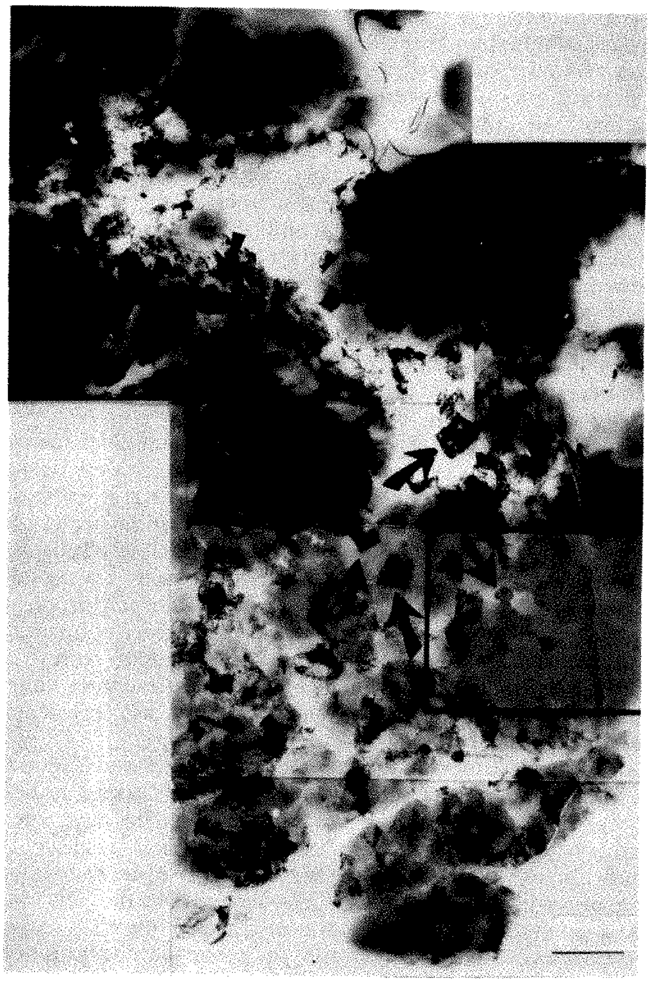

Fig. 1 HVTEM mosaic showing the general shape of test crystals and the contour of the surface. Irregular bright areas are very thin regions or holes in the specimen (~1 μm thick). Arrows point out crystals with characteristically faceted shapes. Tilting in the microscope to bring different crystals into good diffraction conditions shows that most crystals have these shapes. 1,000 kV. Rectangle shows the area of Fig. 2. Scale bar, 5 μm.

By HVTEM, the lack of surface alteration is shown by the smooth outline of the test wall against the coarse 'sparry' calcite which now fills the chambers (Fig. 1). The individual microgranules of the test are equidimensional, 1–5 μm diameter crystals bounded by faces with numerous re-entrant angles (Figs 2 and 3). Note that this microstructure is distinctly different from that produced by recrystallisation of calcite or other monomineralic substances (for example, photomicrographs and electronmicrographs of recrystallised calcite, quartz and olivine are indistinguishable from each other[7–9]). A recrystallised microstructure consists of equant crystals with dominantly planar faces such that the grain boundaries approximate a 'foam' structure produced by equilibration of grain boundary energies. Re-entrant angles are exceedingly rare, and the orientation of crystal faces is unrelated to the crystallography of individual crystals. In these fusulinids, however, the opposite is true; re-entrant angles are common (Figs 2 and 3) and large numbers of crystals have symmetrically related faces (Figs 1 and 2) and faces parallel to internal surfaces (Fig. 3). These features are reminiscent of the miliolid wall structure seen in HVTEM of *Alveolina quoii* d'Orbigny, although in the miliolids the crystals are greatly elongated in one direction (compare with Pl. 9, Fig. 4 of ref. 4). Within the fusulinid crystals and on the boundaries between them, voids are abundant (Fig. 3). These voids range downward in size from ~1,000 Å to <50 Å. Within individual crystals, voids tend to be approximately spherical or ellipsoidal,

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* Present address: Department of Oceanography, University of Hawaii, Honolulu 96822.

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but some of the larger ones are irregular. Grain boundary voids are commonly sharply angular and frequently a tetrahedral gap with perfectly planar faces left between four crystals which did not quite grow together can be seen (Fig. 3*c*). These voids probably contained organic matter originally, but are now empty. In addition to the voids, however, all crystals of the test contain myriads of very small inclusions with images ranging in size from ~100 Å down to the resolution of the microscope, ≈4 Å (Fig. 3*b*). The visibility and apparent size of these inclusions are critically dependent on the diffraction conditions because they produce strain contrast in the host calcite; hence their maximum size may be <100 Å. The inclusions are concentrated on low angle (subgrain) boundaries but are also abundant in regions of perfect crystal. Dislocations are rare in these crystals; some do and others do not have concentrations of inclusions along them.

In contrast to the crystals of the test, the infilling 'sparry' calcite crystals are very coarse-grained, and commonly only a few crystals fill an entire chamber. Voids are rare in these crystals and inclusions are totally absent. Dislocations are locally abundant (Fig. 2) and are occasionally organised into subgrain boundaries; in other areas the dislocation density is very low ($<10^6$ cm^{-2}). In many areas the infilling calcite displays a poorly defined lamellar structure with a wavelength of a few hundred ångströms (arrow, Fig. 2). This latter structure greatly resembles the incipient exsolution lamellae (spinodal decomposition?) common in pyroxenes from igneous rocks[10] and may represent a similar phenomenon.

Alteration of fossils by solution effects or recrystallisation is very common[11]. As a consequence we initially hoped to find some small regions of the samples which might have been preserved through the nearly 300 Myr since their fossilisation. Surprisingly, we find that virtually everything we see seems to be free of modification. We reach this conclusion by the following reasoning: (1) Even on the scale of the electron microscope, external form of the fossils is faithfully preserved; the transition to the infilling calcite is abrupt. (2) The crystal shapes are unlike any polycrystalline calcite ever described; in particular they are unlike extremely fine-grained natural and experimentally recrystallised material[9]. Moreover, except for lack of elongation, these crystals and the voids between them display many of the characteristics of living miliolids[4]; the shapes observed seem to be the result of mutual interference of faceted crystals growing in an isotropic medium—like crystals in an igneous rock. (3) The pattern of voids and minute inclusions in the crystals of the test is distinctly different from that of the infilling calcite, yet absolutely characteristic of the shells of Foraminifera and other organisms[3,4]. (4) The infilling calcite contains a lamellar structure which is not found in the tests, suggesting a different chemistry.

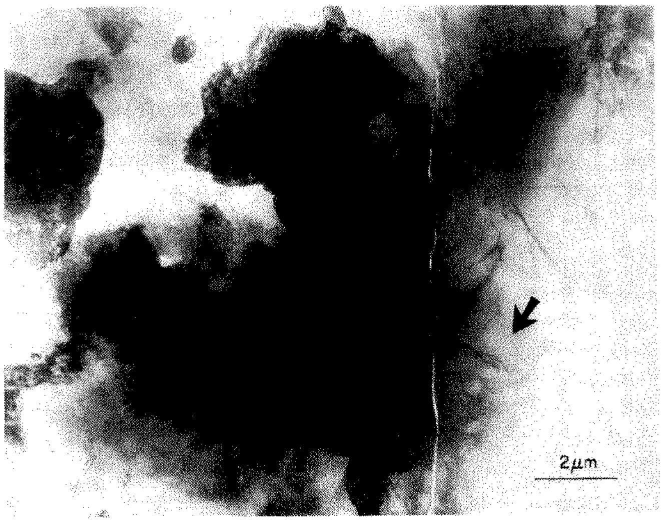

Fig. 2 Detail of portion of Fig. 1 with specimen tilted to a new orientation to show clearly the substructure of the infilling calcite. Note that the crystals of the test appear different than in Fig. 1. This is due to change in the diffraction conditions of individual crystals. Arrow points to lamellar feature in infilling calcite (see text). Scale bar, 2 µm.

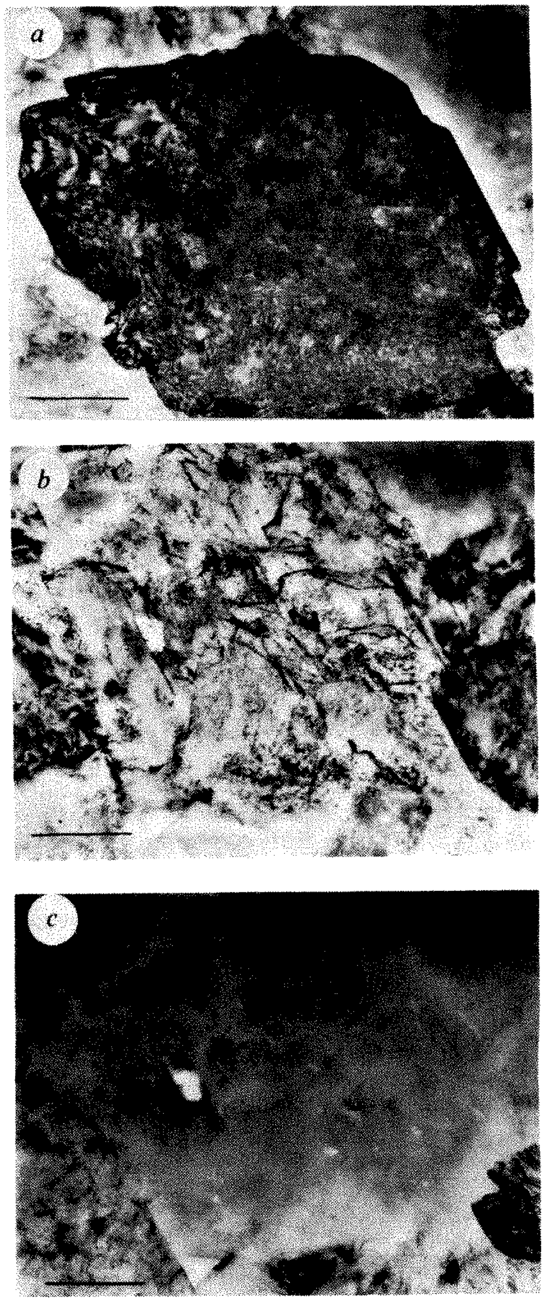

Fig. 3 Detail of typical crystal from interior of test wall. Note planar crystal faces with re-entrant angles. 1,000 kV. Scale bars, 1 µm. *a*, Crystal in strong diffraction contrast. Irregular mottling is caused by elastic distortion of crystal around minute inclusions. *b*, Same crystal, different tilt. Crystal now is in strong contrast only in distorted regions around inclusions. Note internal boundaries parallel to external faces. *c*, Same crystal, tilted to minimise contrast around inclusions. Numerous voids are now visible, including the large triangular one at the lower left where a corner of this crystal meets two others. Note that in detail this void is a triangular pyramid with planar sides.

Of course, a pseudomorphous replacement of the original crystals (calcite or another mineral) could be postulated such that the original, biogenic texture was preserved. This is unlikely because no evidence of such replacement has been found. Moreover, the distribution of inclusions makes such an *ad hoc* hypothesis doubtful because the behaviour of both fluid and solid inclusions during inorganic recrystallisation is well known: inclusions either interfere strongly with grain growth, resulting in a distinct and characteristic modification of the texture, or they are swept out of the interior of grains and concentrated at the boundaries. We do not know of any case where large numbers of very small inclusions remain undisturbed as a grain boundary migrates past them, nor would it be expected.

The similarities to miliolid ultrastructure and the similarity of both to interference structures such as in igneous rocks or metal castings suggest that the individual crystals grew independently in a fluid or fluid-like environment (in this case probably organic matter)[2]. Each crystal grew with its characteristic crystal faces until it came into contact with other crystals. At the point of contact the two crystals would interfere and one or both would lose its crystallographically controlled shape. The preservation of part or all of the crystal form on the majority of crystals indicates that, as in the miliolids, the calcification process did not go to completion; a significant volume remained occupied by organic matter. This is also likely to be the process of calcification of miliolids; it is possible that the different crystal shapes between the two groups are controlled by the chemistry of the organic medium, much as experimental crystal growth can be controlled by adsorption of specific impurities on certain crystal planes.

Although we argue that the basic microgranular test ultrastructure is original, two types of alteration could still be present: (1) Part of the original intercrystalline organic matter may have been replaced by inorganic calcite during diagenesis. We believe that any such replacement must have been minor because there is no general fine-grained matrix between original crystals, and the pattern of inclusions within the original crystals extends to the grain boundaries, making diagenetic overgrowths also unlikely. (2) The chemistry of the test calcite crystals could conceivably have been modified. Towe and Hemleben[11] have shown that fossil miliolids can retain the external form of their original crystals, yet have a much lower Mg content than the crystals of living miliolids. It remains to be demonstrated whether such cation exchange could leave unaltered the pattern of minute inclusions within crystals reported here.

The strong strain contrast around the intracrystalline inclusions indicates that they remain filled. The same effect is apparently seen here as that around the inclusions in living species of Foraminifera and other organisms, and in these latter cases the larger inclusions can be seen to contain organic matter[4]. Unfortunately, we have not found any inclusions in these fusulinids which are large enough to identify organic matter positively within them (the same is true for many modern foraminiferal tests; those that have very small crystal sizes also have very small inclusions). Nevertheless, the evidence suggests that the original contents of these inclusions are still there—locked away and protected inside their calcite 'bottles'—and possibly material could be concentrated from specimens and analysed.

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Genetic heterozygosity in a natural population of *Mus musculus* assessed using two-dimensional electrophoresis

Robert R. Racine & Charles H. Langley

Laboratory of Animal Genetics, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina 27709

Since the introduction of gel electrophoresis to population genetics, estimates of genic heterozygosity at allozyme loci have been made for many organisms[1]. However, attempts to extend the range of proteins surveyed[2–4] have been hampered by concern that there may have been bias in the loci sampled: nearly all proteins for which population data are available are soluble in low-salt extracts and most are enzymes belonging to particular groups such as the nonspecific esterases and phosphatases, various dehydrogenases and the enzymes of the glycolytic pathways and the citric acid cycle. Until recently, there were no techniques for estimating protein variation which were without such bias. Two-dimensional gel electrophoresis[5], however, offered hope of overcoming this bias, and it has proved useful for the resolution of proteins in crude homogenates and for estimating genetic variation[6–9]. We have examined heterozygosity in a wild population of the house mouse (*Mus musculus*) by means of two-dimensional electrophoresis of whole kidney. As we report here, the observed level (2%) is substantially below the level detected by starch gel electrophoresis[10–12]. Our results corroborate similar differences observed in *Drosophila*[6] and man[7,9].

The O'Farrell[5] technique is a method for high-resolution, two-dimensional separation of proteins in polyacrylamide gels. It involves two independent criteria for separation. In the first dimension, denatured proteins are separated according to their molecular weight as assessed by SDS electrophoresis. However, certain questions must be answered before the method can be applied to surveys of genetic variability. Can products of distinct structural loci be distinguished? Can allelic substitutions at a single locus be distinguished? What is the bias of this method with respect to the protein type examined? From O'Farrell's[5] original estimates and from subsequent experiments[13], it is evident that gene products of different loci can routinely be distinguished, and that amino acid substitutions involving residues of different charge will be detected[14,15]. However, there seem to be other, perhaps conformational, differences[6,16] detectable by native electrophoresis (electrophoresis in non-denaturing conditions), or native isoelectric focusing (IEF) that are not detected with O'Farrell's technique. The inherent bias of this technique is towards loci with more abundant gene products, as the detection depends on nonspecific protein staining or labelling.

Figure 1 shows a typical two-dimensional protein pattern from a SDS-urea extract of a mouse kidney. About 100 spots can easily be scored. In our survey, we scored only spots in the

Table 1 Polymorphic loci detected in the Skokholm wild population of the house mouse (*Mus musculus*)

Allele designation	Frequency	Expected heterozygosity
a_1	0.64	0.46
a_2	0.36	
h_1	0.44	0.49
h_2	0.56	
i_1	0.80	0.32
i_2	0.20	

Expected heterozygosity is calculated as $1-(f_1^2+f_2^2)$, where f_1 and f_2 are the frequencies of the number 1 and 2 alleles, respectively.

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central region of the gel, as the *p*H gradient (indicated in Fig. 1) becomes nonlinear at the edges, resulting in loss of resolution in the IEF. We identified three spots showing variation consistent with a simple genetic interpretation[17] (Fig. 2). Protein *a* is isocitrate dehydrogenase (Id-1), which maps on chromosome 1 (ref. 17). The frequencies of the alleles controlling the variant Id-1 spots are shown in Table 1. We were not able to identify the biochemical role of the other two proteins[17]. Table 2 summarises our results and gives the calculated average per-locus heterozygosity together with information on the allozymic variation provided by Berry[10].

We have taken into consideration the fact that a single allele of locus *a* (identified as Id-1) displays two spots on a two-dimensional gel by doubling the expected heterozygosity of locus *a* (Table 1) for calculating our average per-locus heterozygosity. The display of two spots by one allele is probably a result of the long storage time of the kidney tissues (>2 yr, R. J. Berry, personal communication), as we did not observe this on fresh samples[17]. In connection with another study[17], we have made a similar observation with 2-yr-old purified Id-1 enzyme[18]. This enzyme preparation displayed several spots across the slab gel at the appropriate molecular weight (R.R.R., C.H.L. and C.-Y. Lee, unpublished results). Similar multiple spots per allele have been reported[9,14]. The 17 loci known to show genetic variation in these studies exhibit 20 spots on two-dimensional gels[6,9,14,17]. We can therefore estimate that the number of loci surveyed is 85% (17/20) of the number of spots scored. The number of consistently scorable spots (85) probably then represent about 72 individual structural loci.

The threefold reduction in heterozygosity (Table 2) obtained by two-dimensional rather than native electrophoresis on the same mice is striking. This observation is in accordance with studies on *Drosophila*[6] and man[7,9]. The difference probably arises from the ability of two-dimensional gel electrophoresis to

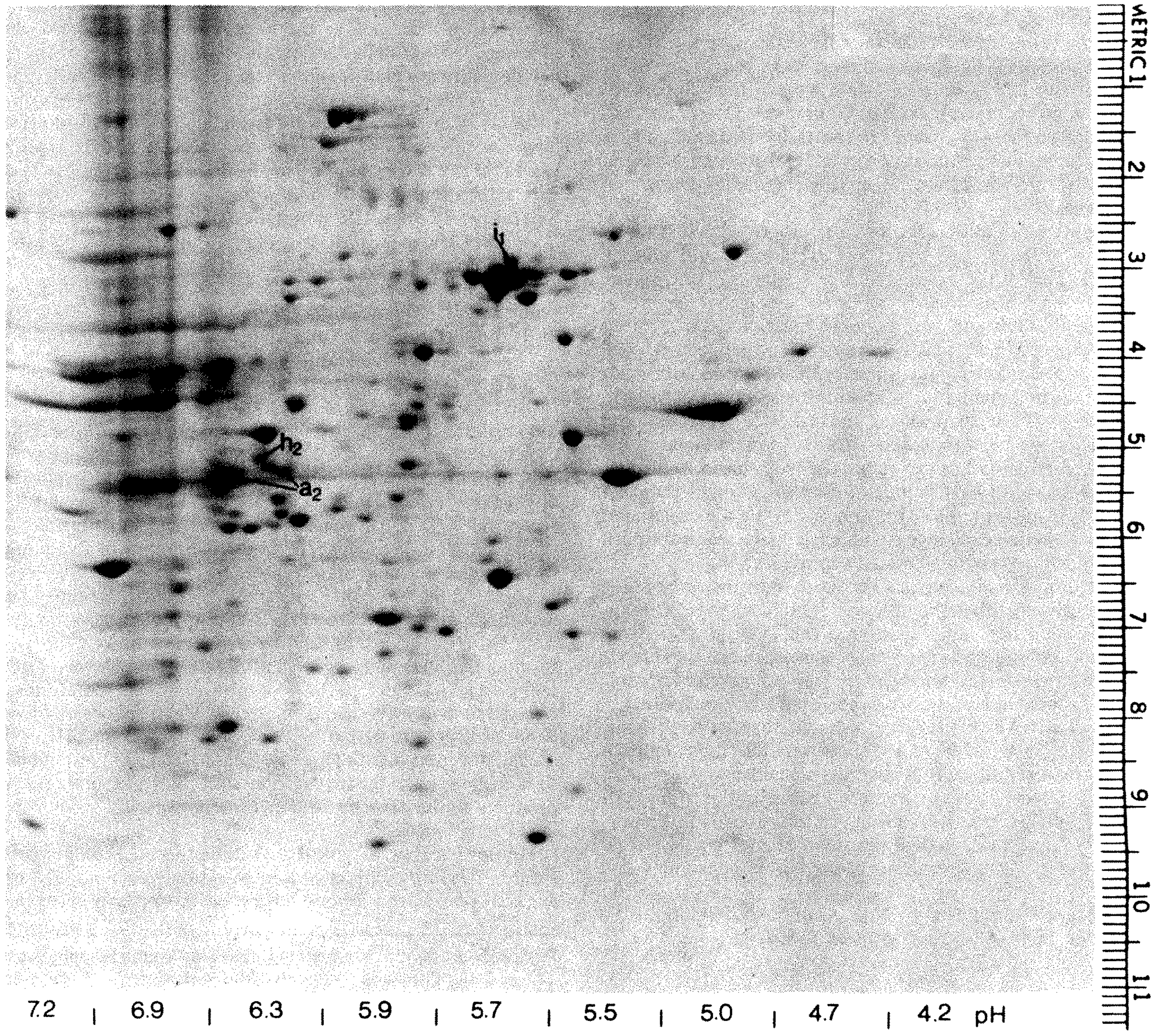

Fig. 1 Two-dimensional gel of a crude kidney homogenate of a wild mouse from Skokholm. *a*, *h* and *i* refer to the three polymorphic spots found. Samples were prepared according to the method described by Wilson *et al.*[20]. Samples (50 μl) were loaded onto the isoelectric focusing (IEF) gels following the procedure of O'Farrell[5]. IEF was carried out in 11.5-cm long Pyrex tubes with an internal diameter of 2 mm, using the same mixture of *p*H 3.5–10 and *p*H 5–8 carrier ampholytes as O'Farrell. SDS slab gel electrophoresis in 10% acrylamide was also carried out as described by O'Farrell. All slab gels were fixed in a mixture of 45% methanol and 10% acetic acid overnight and stained in the same solution containing 0.02% Coomassie brilliant blue R-250. Gels were destained in a mixture of 25% methanol and 7% acetic acid. They were photographed on Polaroid type 55 positive/negative film with a red filter. Throughout, 1% dithiothreitol (Eastman) was used instead of 5% mercaptoethanol. All other reagents were obtained from the same sources[5]. Molecular weights in the slab gels were determined by running in parallel RNase (13,000), chymotrypsinogen (25,000), aldolase (subunit 40,000) and ovalbumin (45,000) (all Pharmacia).

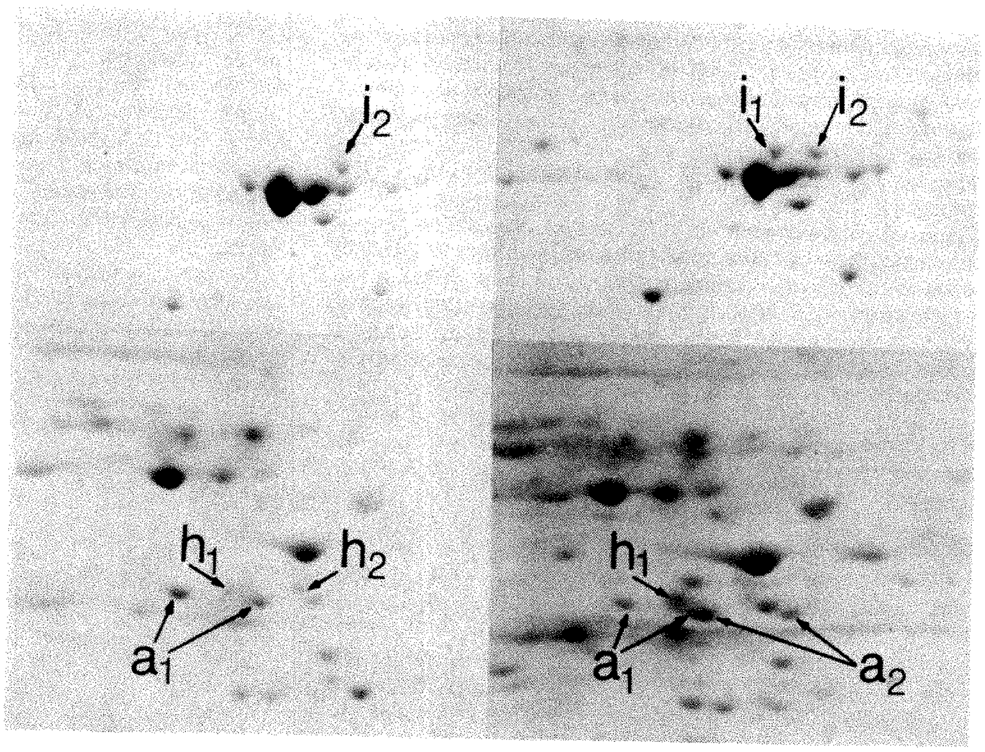

Fig. 2 Two-dimensional gel patterns of the variant regions.

survey previously inaccessible classes of proteins. The two techniques cannot be directly compared, as IEF is not capable of resolving several enzyme variants in *Drosophila* which can be distinguished with standard, one-dimensional methods[16]. Leigh Brown and Langley[6] have compared 10 species of *Drosophila* using α-glycerophosphate dehydrogenase identified on a two-dimensional gel. Several species showed slight differences in mobility in standard starch gel conditions, but on two-dimensional gels only major charge differences (such as the usual slow/fast polymorphism) were detected. Carbamylation experiments[14,15,19], however, show that two-dimensional electrophoresis can resolve all substitutions in the primary structure of a protein which result in a charge difference. The extra variation observed in native, one-dimensional gels is therefore either conformational or post-translational in nature. As the two-dimensional gel technique detects only charge-change substitutions, estimates of total heterozygosity are impossible.

Table 2 Comparison of average per-locus heterozygosity levels between two-dimensional (2-D) and starch gel electrophoresis evaluated in the same wild mice from Skokholm

	2-D gels	Starch gels
No. of loci scored	72 (85)	26
No. of loci polymorphic	3	5
Expected heterozygosity	0.020*	0.172

Number in parentheses indicates the actual number of proteins scored. For further explanations see text. Starch gel data are from an extensive study done by Berry[10].

* Calculated $0.020 = (2 \times 0.46 + 0.49 + 0.32)/85$.

However, if the conditions used in most standard, one-dimensional surveys of allozyme variation are also such as to detect only full charge differences, as is likely, the drop in heterozygosity evaluated by two-dimensional electrophoresis compared with standard electrophoresis, is striking. It might suggest that the most abundant proteins in mouse kidneys represent a group of proteins which are different from and less variable than those which are detected in standard electrophoresis. However, if the proteins identified with the two-dimensional technique are representative of most mouse proteins, the average level of genic heterozygosity is much lower than that previously estimated.

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Identification of specific leech neurones immunoreactive to enkephalin

Birgit Zipser

Cold Spring Harbor Laboratory, PO Box 100, Cold Spring Harbor,
New York 11724

The small endogenous peptides, Met- and Leu-enkephalin, bind to the same specific receptors as opiate analgesics. They, and the larger endorphin peptides, have been widely found in mammals, where they seem to have a significant role in neuronal pathways mediating pain and emotional behaviour. Only recently has enkephalin-like activity been identified in an invertebrate, the earthworm[1], although there is some preliminary evidence for opiate receptors in a marine mollusc[2]. Here I report the detection, by an immunocytochemical technique, of an enkephalin-like moiety which is localised in one of the 400 cells of each posterior midbody ganglion of the leech. The presence of enkephalin-like activity in an identifiable easily accessible neurone of a well characterised nervous system such as that of the leech[3–5] could greatly facilitate elucidation of its mechanism of action.

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Neurones in the refrigerator-stored leech react optimally to Leu-enkephalin antisera (Immuno Nuclear Corporation) at dilutions of 1/5,000 and 1/10,000. The same concentrations of this batch of serum also gave optimal immunocytochemical staining of monkey spinal cord sections (Ellyn Glazer, personal communication), indicating that the immunoreactivity to enkephalin in leech ganglia is of similar strength to that in the mammalian material.

Figure 1*a* shows a series of three successive midbody ganglia from a single leech stained for Leu-enkephalin. Only one cell body at about the same position in each ganglion is immunoreactive. This 30-μm cell body appears on the ventral surface in the posterior half of the ganglion. A stained process leading from this cell body can occasionally be seen in the microscope but is hardly visible in the photographs.

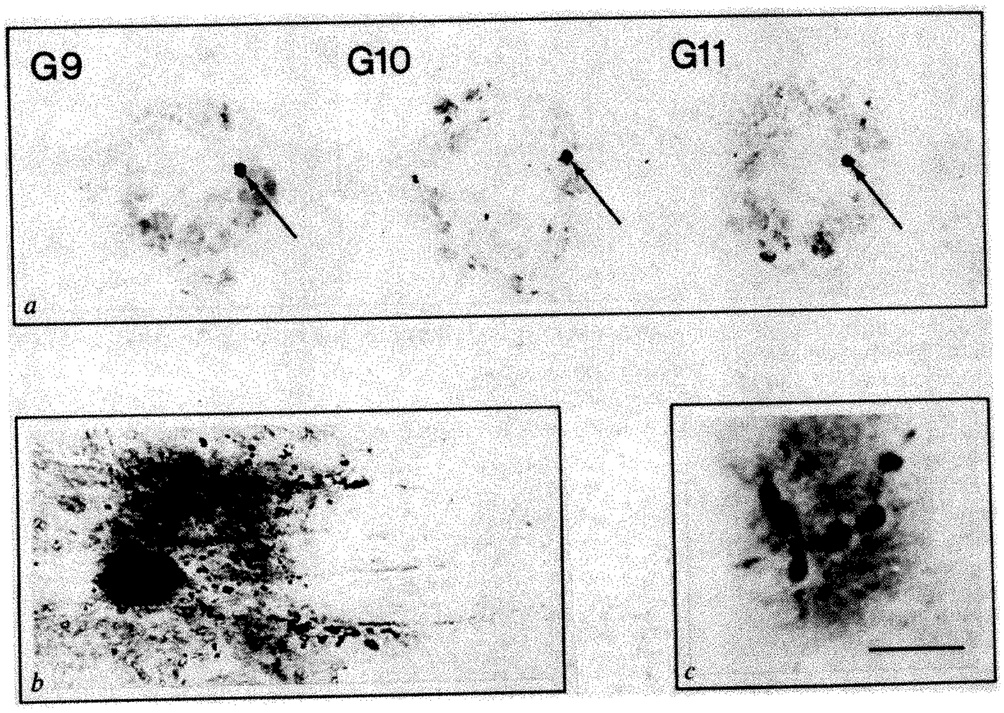

Fig. 1 Enkephalin-immunoreactive cell bodies and neuritic processes in leech ganglia. *a*, A specific cell body in successive ganglia of a *Haemopis marmorata* leech stained by reaction with PAP-coupled Leu-enkephalin antisera. *b*, *c*, The central neuropile showing immunoreactive processes and varicosities. The immunocytochemistry was done on whole ganglia whose ventral capsules were cut to expose the ventrally located unipolar cell bodies. To facilitate diffusion of antibodies across membranes, the 4% paraformaldehyde-fixed ganglia were put through a successive alcohol series into xylene and brought back into buffer before a 2-day incubation with serum containing 0.2% saponin. Calibration: *a*, *b* and *c* are 150, 35 and 13 μm, respectively.

Figure 1*b* and *c* show photomicrographs of the central neuropile of a midbody ganglion at two magnifications. In Fig. 1*b*, a pattern of immunoreactive varicosites is visible. These darkly staining entities at the level of the neuropile strongly resemble nerve terminals of other neurones in the leech[6]. They seem to have a wide size range, with the largest reaching about 4 μm. The individual varicosities are visible at the high magnification of Fig. 1*c*. These varicosities are present in all ganglia, even those in the anterior cord, where cell bodies are generally not stained.

The presence of bound antibody was demonstrated using the peroxidase–antiperoxidase (PAP) procedure developed by Sternberger[7]. Following the incubation with rabbit anti-Leu-enkephalin (Immuno Nuclear Corporation), the ganglia were washed and incubated with goat anti-rabbit IgG (1/20; Cappel) for 30 min, then with the PAP complex (1/250; Cappel) for another 30 min, and finally stained by reaction of peroxidase with 3',3-diaminobenzidine.

Non-immune and Leu-enkephalin-blocked controls were run to confirm that the stained structures were reacting specifically to antibodies against enkephalin. Half ganglionic chains of three different leeches were incubated with non-immune serum of the same dilutions as the enkephalin antisera and neither the 30-μm cell body nor the varicosities were stained, whereas material from the same leeches run in parallel with enkephalin antisera was stained. Leu-enkephalin antiserum was blocked by incubation with Leu^5-enkephalin (Boehringer) at concentrations of 500, 100 and 20 μg ml^{-1}. The 30-μm cell body was not stained over this full 25-fold range. The reaction of the varicosities was concentration dependent; they were not stained when the serum was blocked with Leu-enkephalin at 500 and 100 μg ml^{-1}, but reappeared faintly at 20 μg ml^{-1}.

In ganglia 5, 6 and 7, instead of a single neurone, several cell bodies can show immunoreactive product. Ganglia 5 and 6 innervate the sex organs and are about 80% larger than the typical midbody ganglion; ganglion 7 has no known specific function.

The most significant aspect of this work is that antisera to Leu-enkephalin reacts positively with one specific cell body that maintains the same size and location in successive ganglia within the posterior two-thirds of the midbody ganglionic chain. By analogy with other leech neurones, it is likely that these cell bodies are homologues which can be identified and studied electrophysiologically. It will be interesting to see if the pattern of enkephalin-like distribution observed here with leeches kept in the cold changes when animals in different behavioural states are studied.

The varicosities that show up so prominently with Leu-enkephalin antisera are presumably enlarged synaptic endings with a high peptide content, as they reappeared earlier than the cell bodies when the enkephalin concentration blocking the antiserum was lowered to 20 μg ml^{-1}. Near the head there are ganglia with clearly stained varicosities but no stained cell body, indicating either that not all enkephalin-containing neurones have stained cell bodies, or that some enkephalin-containing terminals originate from cells in other ganglia.

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Reversible events in the transduction process of photoreceptors

K. Hamdorf* & K. Kirschfeld

Max-Planck-Institut für biologische Kybernetik, Spemannstrasse 38,
7400 Tübingen, FRG

In photoreceptors, a latency of many milliseconds elapses between the absorption of a light quantum and the occurrence of the late receptor potential, even for strong light stimuli. Surprisingly, this is much longer than the time necessary for conductance changes such as occur in membranes of neurones or muscles, mediated by chemical transmitters. There are several possible explanations for the long photoreceptor latency. (1) It may be due to properties of the visual pigment molecules. For instance, the temporal coincidence of the occurrence of metarhodospin II with the receptor signal indicates that the meta I-meta II transition might be the trigger for the electrical response in vertebrate photoreception[1]. (2) It may be explained by properties of transport processes. Such a time consuming process could be the diffusion of an internal 'transmitter substance', which diffuses to a 'pore' in the receptor membrane. (3) A third possibility is the time needed to produce and accumulate chemical substances. The light-induced change of the visual pigment molecule might trigger a chemical reaction chain, in which the product of an earlier step triggers the next one. The experiments described here show that a considerable part of the long latency in photoreception is due to processes that are localised at the level of the visual pigment molecule.

Photoreceptors of invertebrates have a stable metarhodopsin (M), which is reconverted into rhodopsin (P) by absorption of light[2,3]. This 'bi-stability' offers the possibility of discriminating between the different hypotheses. In the photoreceptors called R_{1-6} of the fly compound eye the transition of rhodopsin (P) into metarhodopsin (M) is fast (time constant $\approx 100\ \mu s$ at room temperature, 500 μs at 10 °C)[4]. This excludes the possibility that the kinetics of the photometrically determined pigment transition is the time-limiting process. Hypothesis (1) can thus be valid only if properties of the photopigment molecule other than those detectable photometrically are involved. The bi-stability of the visual pigment system of the fly photoreceptors allows one to reconvert $M \rightarrow P$ within the latency of the response. If hypotheses (2) or (3) were correct, an irreversible process should have been initiated within a fraction of a millisecond, and the reconversion of $M \rightarrow P$ should not affect the response. These hypotheses therefore can be rejected if it can be demonstrated that the reconversion reduces the response.

As will be seen below, a successful experiment can be performed only in receptors in which the rhodopsin content has been reduced to low concentration.

Figures 1 and 2 show the amplitude of the receptor response to light flashes as a function of intensity. With strong stimuli the response amplitude becomes saturated.

In the visual pigment system of receptors R_{1-6} of the blowfly, blue light shifts a maximal fraction of P into M, red light a maximal fraction of M into P[5,6]. With short double flashes (blue followed by red), P can be first converted to M and then back again within the latency of the response. The effect of such double flashes on the prolonged depolarising after-potential and the 'pupil response' in *Calliphora* has been described by Muijser and Stavenga[7].

Figure 1*c* shows responses to a blue flash and to the sequence blue–red. It is obvious that, compared with the single blue flash, the second red flash reduces the response amplitude. This result seems to be paradoxical because more absorbed light quanta lead to a smaller response. This paradoxical result is obtained only with the sequence blue–red, whereas the sequence red–blue leads to the same response amplitude as a single blue flash. Blue–blue double flashes always result in an increase of the response amplitude as long as the response has not been saturated by the first flash, and if the second flash is not given too late.

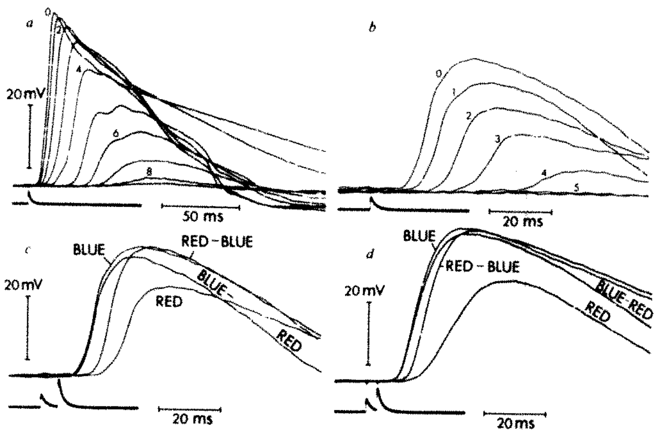

Fig. 1 Intracellularly recorded responses of photoreceptors (type R_{1-6}) of *Calliphora* female, chalky, to light flashes. Monitor, lower trace; temperature, 10 °C. *a*, Fly with high rhodopsin content in the receptors. Relative flash intensities were changed from 10^0 to 10^{-9} (numbers indicated on the traces). *b*, Fly with rhodopsin content reduced to 1/1,000 of normal. *c*, Same cell as that used in *b*. Stimulus, blue and red single flashes; blue–red and red–blue double flashes with 5 ms delay, all of maximal intensity. Note that the longer latency of the sequence red–blue is due to the fact that the blue flash occurs as the second one. *d*, Same stimulus programme as used in *c*, delay between double flashes 3 ms; records from another fly.

The amplitude to the sequence blue–red corresponds to an amplitude to a single blue flash reduced to some 25% of maximal intensity. This can be seen from the intensity amplitude function (Fig. 2). The response is not completely abolished for two reasons. First, for technical reasons, the second red flash was not bright enough to reach equilibrium completely. Hence, from the M created by the first flash, only two-thirds are shifted back into rhodopsin. Second, the red flash by itself shifts a small amount of P into M. From the amplitude of the red response this amount can be estimated to be 1–2% (see arrow in Fig. 2).

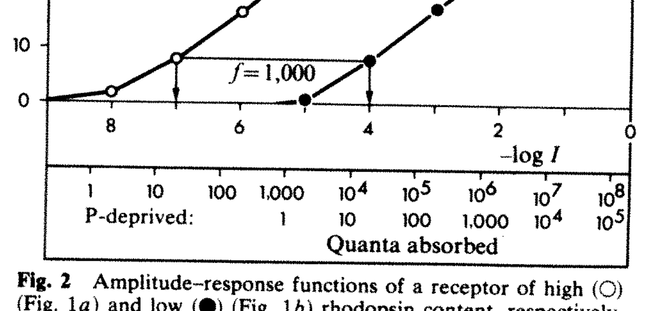

Fig. 2 Amplitude–response functions of a receptor of high (○) (Fig. 1*a*) and low (●) (Fig. 1*b*) rhodopsin content, respectively. Abscissae, –log relative intensity; quanta absorbed. Ordinate, amplitude of receptor potential in mV. Arrows indicate amplitudes of responses from Fig. 1*c* to blue, blue–red and red flashes. *f* values indicate sensitivity factors.

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* Permanent address: Ruhr-Universität-Bochum, Lehrstuhl für Tierphysiologie, Postfach 2148, 463 Bochum-Querenburg, FRG.

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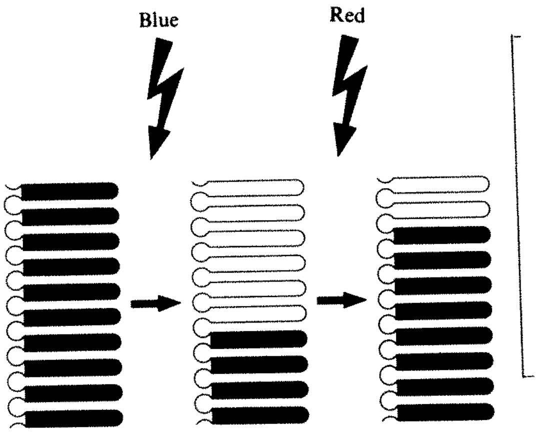

Fig. 3 Microvilli (shown schematically) with visual pigment in either rhodopsin (P) (black) or metarhodopsin (M) conformation. The first blue flash shifts some 60% of P into M; after the second red flash only 20% of the pigment is left as M.

If we want to stop the conductance change induced by the P→M conversion, we must hit the same molecules with the second red flash. The probability of doing so is high only at very high flash intensities which already saturate the cell. Hence, a possible reduction of the response to the blue–red double flash cannot be detected at normal visual pigment content of the receptors. To remain below the saturation of the response we must reduce the pigment content to such a degree that less than 10^5 pigment molecules can be hit; this means that the pigment concentration must be <1 ‰ of normal[8]. (The reduction in P content to <1‰ of normal was achieved by raising flies in special conditions developed by Schwemer[9].) In this condition, the sensitivity of the receptors is markedly reduced (Fig. 2), but, as has been shown, the double-flash experiment can be successfully performed (Fig. 1*c*, *d*). Quantitatively we obtain the following situation. The first blue flash shifts 60% P into M. The second red flash shifts back 65% of those M molecules, so that 20% remain (Fig. 3). These values have been derived from photometrical measurements in the experimental set-up used for the electrophysiological experiments[10].

If the pigment content is sufficiently reduced, the amplitude to the blue–red double flash is always smaller than that to the blue flash alone. However, the amplitude reduction was often not as prominent as that shown in Fig. 1*c*, and corresponded to a loss in sensitivity by a factor of only 2 to 3.

Following the conversion of P to M ($\tau \approx 500$ μs, 10 °C), there remains a period of 3–5 ms during which reconversion of M to P more or less completely abolishes the effect of the initial P→M conversion (Fig. 1*c*, *d*). During this period no irreversible transmitter release or chain reaction could have been initiated. It is true that there remain a further 3–4 ms before the onset of the response during which such processes may become important. What has been clearly shown is the existence of a distinct, time-limiting process in transduction localised at the level of the pigment molecule and lasting a few milliseconds.

Furthermore, we are able to decide whether the response of the receptor is due to the total number of P–M conversion or to the number of M molecules remaining after the stimulus. Because, in both flash sequences (red–blue; blue–red), the number of P–M conversions is the same whereas the number of M molecules remaining is different, it must be the M molecules remaining after the flashes which trigger the conductance change.

As we are able to stop the sequence of events within 3–5 ms, the action of the freshly created M must last longer than 3–5 ms in order to trigger the response. After having excited the membrane, the M molecules remain as thermostable M without any further excitatory effect. Therefore, it is the newly created M which excites the membrane. This M has been called 'energy rich' in the photoreceptor membrane model developed from other experimental evidence[11].

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Photoreceptor signals at visual threshold

C. A. Dvorak*, A. M. Granda† & J. H. Maxwell

Institute for Neuroscience and Behavior, University of Delaware, Newark, Delaware 19711

Electrical responses of cone photoreceptors in the retina of the freshwater turtle have been characterised for flashes and steps of light in darkness and in the presence of background light[1–4]. These intracellular measurements have been combined with the behavioural increment threshold curve to yield an estimate of 5–10 μV for the signal developed in a cone when the turtle can just detect an increment flash[5]. The signal developed when the cones under the stimulus image are dark-adapted is of interest, for its measurement would help to explain how known physiological processes subserve visual detection for a variety of photic conditions. The effective quantal absorption of dark-adapted, red-sensitive cones of *Pseudemys scripta elegans* for a stimulus that the turtle can just detect is reported here. By combining this result with previous electrical measurements on red-sensitive cones of this species, an estimate of 35–70 μV is obtained for the signal developed in a dark-adapted cone at behavioural threshold. This larger signal required for detection of a flash in darkness is of particular interest in view of the recent observation[6] that the intrinsic noise of turtle cones in darkness is larger than that of illuminated cones.

Details of the experimental apparatus and the behavioural paradigm have been presented elsewhere[1,7]. Basically, the animal was placed in an experimental chamber with its head positioned so that the stimulus image fell on the central linear streak of the retina. The turtle was trained to avoid an electric shock by pulling in its head whenever it detected a flash of light. An 'up-down' tracking procedure was used to determine the threshold with mean 95% confidence intervals of about 0.1 log units. The animal was dark-adapted for 45 min before presentation of a 2.35° diameter stimulus for 125 ms. As these values represent the approximate critical area and the exact critical duration for white (tungsten) light (see Figs 31 and 32 in ref. 1), essentially complete spatial and temporal summation occurred. The radiant emittance M_e of the source was determined as a function of wavelength using a calibrated thermopile, and for the attenuated stimulus was 2.1×10^{-8} J cm^{-2} s^{-1}. From Lambert's law the radiance L_e of the stimulus was 6.5×10^{-9} J cm^{-2} sr^{-1} s^{-1}. The retinal irradiance E_e can be determined[8] from the relation $E_e = \pi L_e$ (n.a.)^2 for a numerical aperture (n.a.) that is equal to $nd/2p$ for refractive index n, entrance pupil diameter d and posterior focal length p. We used values of $n = 1.34$ and $p = 6.40$ mm from the schematic eye of *Pseudemys*, which has an actual pupil diameter of 1.74 mm (data of D. P. M.

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* Present address: Xerox Corporation, Image Structure and Perception Area, Webster Research Center, Building 114, Rochester, New York 14644.

† To whom reprint requests should be addressed.

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Northmore in ref. 1). The entrance pupil is located 0.21 mm anterior to the actual pupil, and was determined to be 1.95 mm in diameter using the corneal magnification factor, which was calculated to be 1.12. This agrees fairly well with the approximate 10% reduction of apparent pupil size when the animal is placed in water, thereby cancelling the refractive power of the cornea. The resultant numerical aperture is 0.203, which, when substituted into the relation for the attenuated retinal irradiance, yields $8.6 \times 10^{-18}\ \mathrm{J}\ \mu\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. When the neutral density of the optical media (0.04) (ref. 9) is taken into account, this figure is reduced to $7.9 \times 10^{-18}\ \mathrm{J}\ \mu\mathrm{m}^{-2}\ \mathrm{s}^{-1}$. The fraction of this just-detectable light at the level of the retina that is effective in terms of the red-sensitive cones was determined by numerically integrating the product of the mean spectral sensitivity of the cones and the spectral distribution of the light, then dividing by the integrated spectral energy of the light. The resulting value is 0.52. This reduces the retinal irradiance to $4.1 \times 10^{-18}\ \mathrm{J}\ \mu\mathrm{m}^{-2}\ \mathrm{s}^{-1}$, which represents the total energy in the stimulus to which red-sensitive cones are sensitive irrespective of wavelength. The optimal wavelength for both the cone sensitivity[2] and the light spectrum is 633 nm, and by weighting the energy at this wavelength a conversion to quanta can be made with a result of 1.6 quanta $\mu\mathrm{m}^{-2}$ per flash. The mean cross-sectional area of a 3.0-μm diameter oil droplet in a paracentral, red-sensitive cone of *Pseudemys*[10] is 7.1 $\mu\mathrm{m}^2$, yielding an average of 11.4 quanta per cone per flash based on the area of the oil droplet. This value is an estimate of the minimum quantal catch by the cone, as oil droplet area is on the average only half the area of the inner segment, according to measurements made prior to the droplet in peripheral cones[11]. Thus, if twice as much light were collected by the cone in view of this possible twofold difference, this value could be as high as 22.8 quanta per cone per flash. Approximately 12% of these 11.4–22.8 mean incident quanta are effectively collected, absorbed and transduced by the outer segment of a red-sensitive cone[11], so on the average 1.4–2.8 quanta are effectively used by each red-sensitive cone at the behavioural threshold. Since each effective photo-isomerisation in a peripheral red-sensitive turtle cone yields a hyperpolarisation of the membrane potential of about 25 μV (ref. 2), an average signal of between 35 and 70 μV is developed in each red-sensitive cone in response to a flash of light that the dark-adapted animal can just detect. An assumption made in arriving at this range is that paracentral, red-sensitive cones also develop approximately 25 μV per effective photo-isomerisation when a large number of adjacent cones are illuminated, as was the case in the determination of the response of peripheral cones.

For the data used in this analysis the results provide an estimate of what is probably a minimum range of effectively utilised quanta: the most sensitive electrophysiological and behavioural data available have been incorporated in our calculations. The use of any other data, such as larger red-sensitive cone diameters for measurements[10,11] taken from the peripheral retina of *Pseudemys*, would yield a larger estimate. To assure that no substantial error was introduced due to the use of white (tungsten) light, we also determined thresholds for red light by inserting a colour filter (Kodak no. 26) in the light path. Because of the decrease in total light available to the red-sensitive cones, the neutral density had to be decreased by 0.15 units in order to obtain an equivalent threshold. Of the red light thus presented we calculated that a difference of about 0.14 log units existed between the two stimuli in terms of their effectiveness in stimulating red-sensitive cones, in comparison to the 0.15 units found experimentally. Similar thresholds for the two stimuli are actually expected in view of the long-wavelength spectral bias of both the animal's sensitivity and the energy of either stimulus. Within experimental error (0.01 density unit), then, we have a determination of threshold using red and white lights that strongly suggests that the animal is using its red-sensitive cones to detect the spectral energy of both stimuli.

The estimate of 35–70 μV for the signal range of the dark-adapted, red-sensitive cones at threshold is several times the 5–10 μV estimate for red-sensitive cones mediating an increment threshold detection. Measurements of intrinsic membrane noise of red-sensitive cones of *Pseudemys* are up to four times larger in darkness than in light, depending on the extent of interreceptor coupling[12]. Thus the respective amplitudes of signals and noise underlying detection in darkness and in light have the appropriate relationship to suggest that the observed visual thresholds could be the result of a form of signal-to-noise discrimination of neural events that originate in the photoreceptors.

Our results indicate that each red-sensitive cone under the 2.35° stimulus spot effectively uses at least one photon, and possibly two. Under the conditions for which thresholds were obtained it would seem to matter very little whether or not the photoreceptors were electrically coupled in terms of their signals, for they would develop about the same photovoltage in either case, since a large number of adjacent cones are on the average absorbing the same number of photons. However, since coupling reduces noise in a given cone[12], the net effect of coupling would be to improve the signal-to-noise ratio during the detection of light under any condition for which a large number of coupled cones are stimulated.

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Endocrine differentiation of the fetal rabbit ovary in culture

Fredrick W. George & Jean D. Wilson

Department of Internal Medicine and Eugene McDermott Center for Growth and Development, University of Texas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, Texas 75235

Although virilisation of the male fetus is accomplished in large part by the secretion of testosterone from the testis during embryogenesis[1], little is known about the function, if any, of the fetal ovary in female embryonic development. On the basis of studies in rabbits, in which castrate fetuses developed as phenotypic females, Jost concluded that the ovary does not have an active role in sexual differentiation[2]. However, we have demonstrated that the onset of oestrogen formation in the fetal ovaries of rabbit and man occurs at approximately the same time that the fetal testis develops the capacity to form androgens[3,4], and Mauleon *et al.*[5] have demonstrated that the fetal sheep ovary secretes 17β-oestradiol before the onset of testosterone synthesis by the testis. These findings suggest that ovarian function is important during embryonic development. In the fetal rabbit, the onset of testosterone synthesis in the testis coincides with the appearance of 3β-hydroxysteroid-$\Delta^{4,5}$-isomerase activity[3,6,7]. The mechanism regulating the time of appearance and the amount of this activity remains unclear, although the enzyme develops in fetal gonads cultured in chemically defined medium[7], suggesting that the regulation of testosterone synthesis is intrinsic to the fetal testis. To gain insight into the factors responsible for the onset of endocrine function of the ovary we have compared the enzymatic development of embryonic rabbit ovaries and testes in organ culture. The results indicate that the enzymatic differentiation of fetal ovaries is also independent of hormonal induction and occurs in culture at the same time as endocrine differentiation of the fetal ovary *in vivo*.

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On day 16 of gestation (the day of observed copulation was designated day 0), New Zealand white rabbits were killed, the embryos were removed aseptically, and the gonads with adjacent mesonephros were dissected from the embryos. Pairs of gonads from individual embryos were cultured at 37 °C in Serumless Medium (Gibco) in a humidified atmosphere of 5% CO_2 in air in separate wells (1.6 cm diameter) of a Linbro plate (Flow Laboratories). Media were changed at 48 h, and fetal gonads and media were collected at the times indicated in the figure legends. The genetic sex of each embryo was determined by staining the amniotic membrane with thionin to show the presence or absence of sex chromatin by the method of Klinger and Ludwig[8]. All gonads in which corresponding amniotic membranes were sex chromatin positive were designated ovaries.

To assess the enzymatic differentiation of fetal testes and ovaries we measured the conversion of 1,2-^{3}H-dehydroepiandrosterone (54.7 Ci mmol^{-1}) to androstenedione, testosterone and 17β-oestradiol in a total volume of 0.2 ml Serumless Medium. Depending on the developmental stage, two to eight fetal ovaries or one to eight fetal testes (representing 0.05–0.1 mg protein) were used in each incubation. After incubation, appropriate ^{14}C-labelled steroids were added in trace amounts to assess recovery, and the samples were extracted and chromatographed as before[4]. Protein was determined by a modification[7] of the method of Lowry *et al.*[9]. Each of the steroids isolated by column chromatography was purified through several thin-layer chromatographic steps, and the authenticity of the isolated products was confirmed by recrystallisation. In addition we measured by radioimmunoassay[10] the 17β-oestradiol secreted into the media from ovarian and testicular cultures.

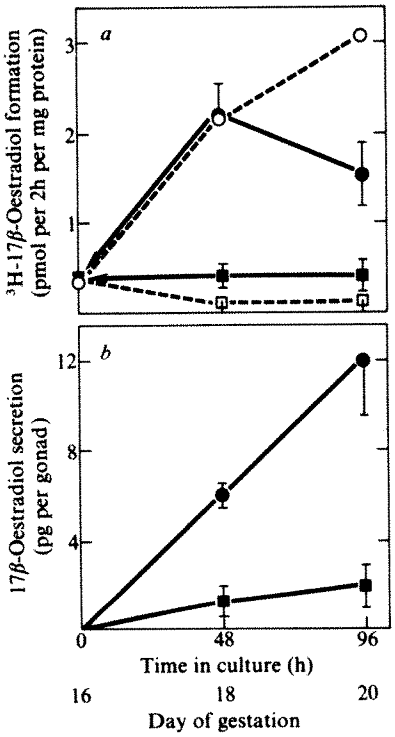

Fig. 1 Developmental pattern of 17β-oestradiol formation and secretion by cultured and *in vivo* control gonads. *a*, ^{3}H-17β-oestradiol synthesis. Cultured fetal gonads were collected 48 and 96 h after the initiation of cultures, dissected free of mesonephric tissue, and incubated with 5 μM 1,2-^{3}H-dehydroepiandrosterone as described in the text. The ^{3}H-17β-oestradiol formed during incubation was purified as before[4]. Rates for *in vivo* control gonads are expressed as the mean of two observations, and the values for cultured gonads are expressed as the mean ± s.e.m. for four observations. ○, *In vivo* control ovaries; □, *in vivo* control testes; ●, ovaries; and ■, testes cultured from day 16 of gestation. *b*, 17β-oestradiol secretion into the culture medium. Media from either ovarian or testicular cultures were pooled separately when the media were changed (48 or 96 h). Each pooled sample was extracted with 5 volumes of ethyl ether and chromatographed on Celite columns as before[3]. Aliquots corresponding to 25% of the column eluates for 17β-oestradiol were assayed by radioimmunoassay[10]. Each sample represents the pooled media from 6–15 pairs of gonads, and the results are expressed as the mean and range of two experiments. ●, Ovaries; ■, testes.

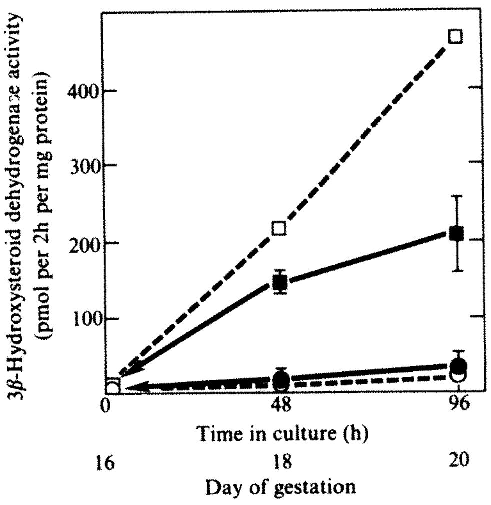

Fig. 2 3β-Hydroxysteroid dehydrogenase activity in cultured and control fetal rabbit gonads. 3β-Hydroxysteroid dehydrogenase activity was estimated by assessing the amount of androstenedione plus testosterone formed in the incubations of fetal gonads with 1,2-^{3}H-dehydroepiandrosterone described in the text and the legend to Fig. 1. ○, *In vivo* control ovaries; □, testes; ●, ovaries; and ■, testes cultured from day 16 of gestation. Data for *in vivo* control gonads represent the mean of two experiments, and data for the cultured gonads represent the mean ± s.e.m. of four observations each.

At day 16 of gestation neither fetal ovaries nor fetal testes synthesised 17β-oestradiol from ^{3}H-dehydroepiandrosterone (Fig. 1*a*). However, after 48 h in culture, fetal ovaries developed the capacity to form 17β-oestradiol at a rate equal to that in control ovaries of corresponding age (2.2 pmol per 2 h per mg protein in cultured ovaries; 2.1 pmol per 2 h per mg protein in day 18 *in vivo* control ovaries). The rate of 17β-oestradiol formation in cultured ovaries declined by 96 h to 1.15 pmol per 2 h per mg protein but remained substantially greater than the rate of formation in both cultured and day 20 *in vivo* control testes. Furthermore, after 48 and 96 h in culture, ovaries secreted four to six times more 17β-oestradiol per gonad into the medium than did testes (Fig. 1*b*), indicating that the observed enzymatic differentiation *in vitro* reflects a true functional oestrogen synthesis similar to that which occurs *in vivo*[10].

3β-Hydroxysteroid dehydrogenase activity was estimated by measuring androstenedione and testosterone formation from 1,2-^{3}H-dehydroepiandrosterone in the same fetal tissues used for the assessment of 17β-oestradiol formation. At the time of explantation (day 16 of gestation) 3β-hydroxysteroid dehydrogenase activity was low in both ovaries and testes (<3 pmol per 2 h per mg protein). There was a progressive increase in 3β-hydroxysteroid dehydrogenase activity in both ovaries and testes throughout the 4-day culture period (Fig. 2), although the final rate was greater in testes than ovaries (209 and 37 pmol per 2 h per mg protein, respectively).

Although the histological and enzymatic differentiation in culture of the fetal testis of rat[11] and rabbit[7] have been described before, our study shows for the first time the enzymatic differentiation of the fetal ovary in culture. ^{3}H-dehydroepiandrosterone was used as substrate to enable us to measure simultaneously the development of the rate-controlling enzyme for testosterone synthesis (namely 3β-hydroxysteroid dehydrogenase-$\Delta^{4,5}$-isomerase) and the capacity for 17β-oestradiol synthesis in the fetal rabbit gonad[6]. Although this procedure may underestimate the enzymatic capacity for oestrogen synthesis, the fact that the secretion of 17β-oestradiol into the medium by cultured ovaries parallels the increase in oestrogen formation from ^{3}H-dehydroepiandrosterone indicates that the differences in oestrogen formation between the fetal ovary and testis are real. Furthermore, similar measurements were obtained after short term incubation of fetal gonads when a saturating concentration of ^{3}H-testosterone was used as substrate for oestrogen synthesis[3].

The fact that endocrine differentiation of the fetal rabbit ovary, as well as the testis, occurs in organ culture at the same time that the process occurs *in vivo* suggests that the differentiation is inherent to the gonads and not controlled by extragonadal factors. Because enzymatic differentiation of the fetal ovary occurs earlier than would be predicted on the basis of histological criteria in rabbit[3], man[4] and sheep[5] it is possible that the onset of 17β-oestradiol synthesis is a prerequisite for other events in ovarian differentiation. If that is true, the genetic composition of the female may be responsible for the development of endocrine function in the female embryo, and the endocrine function in turn may be responsible for the histological differentiation of the ovary. Regardless of any endocrine implications, the demonstration that enzymatic markers for fetal ovaries and testes develop *in vitro* will be useful in the study of factors controlling gonadal differentiation.

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Lactogenic receptor regulation in hormone-stimulated steroidogenic cells

Terry F. Davies*, Mohan Katikineni, Vivian Chan, James P. Harwood, Maria L. Dufau & Kevin J. Catt†

Endocrinology and Reproduction Research Branch, National Institute of Child Health and Human Development, NIH, Bethesda, Maryland 20205

Receptor sites for lactogenic hormones such as prolactin (PRL), human growth hormone (HGH), and placental lactogens, are widely distributed in mammalian tissues, including mammary glands[1], steroid-secreting cells of the adrenal, testis, and ovary, and target cells of steroid hormone action such as liver, prostate, and kidney[2,3]. Although the biological functions of lactogen binding sites remain uncertain, a relationship between prolactin and lipoprotein metabolism is implied by the occurrence of prolactin receptors in steroidogenic cells of the gonads and adrenal, and by the ability of prolactin to increase esterified cholesterol in the testis[4]. Recently, loss of testicular prolactin receptors has been observed following elevation of circulating luteinising hormone (LH) concentrations by the gonadotropin releasing hormone (GnRH) and its agonist analogues[5,6]. The hormone dependence of lactogen receptor sites in steroid-secreting cells was further analysed in rat testis, ovary, and adrenal glands after treatment with the respective trophic hormones, gonadotropin and ACTH. In each of these tissues, rapid and transient loss of lactogen receptors was observed after trophic hormone stimulation. These findings indicate that increased turnover of lactogen receptors is an important component of the target-cell response, and suggest that prolactin receptors might be involved in the transport of lipoprotein precursors for steroid biosynthesis.

Previous studies have shown that treatment with exogenous gonadotropins such as ovine LH (oLH) or human chorionic gonadotropin (HCG) causes marked and prolonged reduction of testicular LH receptors[7–9]. This process of receptor depletion, commonly referred to as down-regulation, is analogous to the effects of other peptide hormones, such as insulin and growth hormone, on their specific receptor sites[10,11]. We have recently observed that lactogen receptors in the testis initially undergo changes in parallel with those of LH receptors after gonadotropin treatment. An early brief rise in both receptor sites is followed by a marked and transient decrease of lactogen receptors after administration of oLH or HCG[12]. An example of the concomitant loss of testicular LH and lactogen receptors after treatment with gonadotropins is shown in Fig. 1. The lactogen receptors were decreased by 70% at 24 h, and returned to normal values at 48 h, whereas LH receptors fell rapidly and remained low for up to 96 h. The reduction in lactogen binding followed the peak of testosterone secretion, which normally occurs within 4 h after administration of either GnRH or exogenous gonadotropin. The disappearance of lactogen receptors was not due to occupancy of the binding sites by endogenous hormone, since no significant increase in serum prolactin was detected by radioimmunoassay after LH treatment. In addition, no change in degradation of labelled HGH was observed in testis fractions prepared from gonadotropin-treated rats.

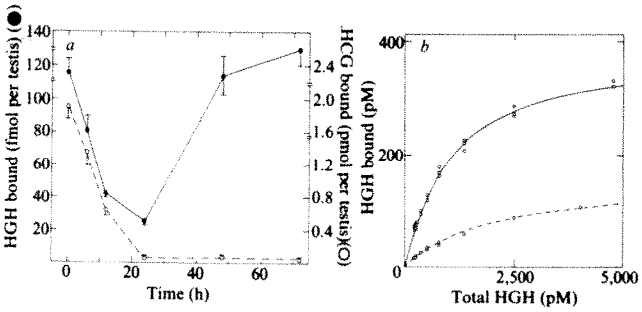

Fig. 1 *a*, Transient loss of lactogenic hormone binding sites in adult rat testes stimulated *in vivo* by administration of 20 µg HCG. A 15,000*g* fraction of testis homogenate was analysed for receptor binding capacity with ^{125}I-HGH and ^{125}I-HCG to quantitate lactogenic and LH receptors, respectively[6,20]. The use of HGH tracer to measure lactogen receptors was based on the ability of HGH to bind specifically to receptors for prolactin and other lactogens[1]. In this and subsequent figures, each point represents the mean ± s.e. of data from groups of six rats. The mean ± s.e. for the control animals throughout the study are shown in double horizontal bars on the vertical axes. *b*, Saturation curves for ^{125}I-HGH binding to 15,000*g* fractions of homogenates from control testes (continuous line) and testes removed 24 h after administration of 100 µg oLH (broken line).

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* Present address: Division of Endocrinology, Mount Sinai School of Medicine, New York, New York 10029.

† To whom correspondence should be addressed at Room 12N-202, Bldg 10, National Institutes of Health, Bethesda, Maryland 20205.

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Table 1 Insulin receptors in the rat ovary during stimulation with HCG

Time after injection with HCG	Insulin receptors (fmol per mg protein; mean ± s.e.)
0 (control)	38.8 ± 0.35
6 h	37.4 ± 1.5
12 h	37.9 ± 7.9
24 h	35.6 ± 0.3
48 h	27.4 ± 4.5

Primed rats were injected subcutaneously with 20 μg of HCG and groups of three rats were sacrificed at the times indicated. Pairs of ovaries were assayed for insulin receptor sites using ^{125}I-insulin. Two aliquots in duplicate of the 15,000g pellet of ovarian membranes were incubated for 16 h at 4 °C with 1.27×10^{-10} M insulin, specific activity 1,325 c.p.m. per fmol. Similar aliquots were incubated in the presence of 5×10^{-7} M unlabelled insulin to determine nonspecific binding.

In the luteinised rat ovary, similar changes in lactogen receptors were observed after gonadotropin treatment. When immature rats, primed with pregnant mare serum gonadotropin (PMSG) and HCG to induce luteinisation of the ovaries[13], were treated with 20 μg HCG, the rapid and marked fall in LH receptor capacity was consistently accompanied by a decrease in binding of ^{125}I-HGH. This decrease in lactogen sites occurred earlier than in the testis and was again coincident with LH receptor loss between 6 and 24 h. The lactogen receptors had returned to normal at 48 h, whereas marked loss of LH receptors persisted for 3 d (Fig. 2). During these gonadotropin-induced changes in lactogen receptors, there was no alteration in the concentrations of two other luteal cell receptors, for insulin and β-adrenergic ligands. As shown in Table 1, insulin receptors in the luteinised ovary remained constant after HCG treatment that caused marked decreases in LH and lactogen receptors. There was a similar lack of effect of HCG treatment on β-adrenergic receptors, which have recently been shown to be functionally coupled to adenylate cyclase and progesterone secretion in the luteal cell[14].

Gonadal lactogenic hormone receptors, like those for LH, are located on the steroidogenic cells of the testis[15] and ovary[16]. The common location of testicular LH and prolactin sites, and the similarity in their rates of depletion after GnRH or LH/HCG treatment, suggest that down-regulation of the two receptors is coordinated after gonadotropin stimulation, and that the two receptors could be physically related within the target cell membrane. Although the LH and prolactin sites are highly specific for the respective ligands, the molecular weights of the two receptors are similar after detergent solubilisation (about 200,000)[17,18]. This was further examined by simultaneous gel filtration of the two binding sites on 6% agarose after extraction from testis interstitial particles with 1% Triton X-100. Subsequent binding assay of the free receptors in each fraction with ^{131}I-HCG and ^{125}I-HGH revealed two coincident peaks of hormone-binding activity with K_{av} of 0.31, consistent with a molecular radius of about 6.8 nm for each of the detergent-solubilised hormone receptors.

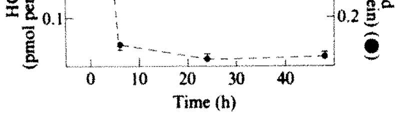

Fig. 2 Binding of lactogen (HGH) (○) and gonadotropin (HCG) (●) to the 15,000g fraction of homogenates from luteinised rat ovaries after subcutaneous treatment with 20 μg HCG given 8 d after priming with PMSG and HCG. Binding assays were performed as previously described[6,13].

In the adrenal cortex, prolactin receptors have been localised to the reticularis and fasciculata zones[19]. Following treatment of male rats with corticotropin (ACTH$_{1-24}$), we observed a marked reduction in adrenal lactogen binding between 6 and 12 h after injection of the hormone (Fig. 3). Although the return of adrenal receptors was more rapid than in the testis and ovary, the initial phase of receptor loss was again subsequent to the peak of steroid (corticosterone) secretion, which usually occurs within 60–90 min of ACTH administration.

The decreased binding of HGH tracer by steroid-secreting cells of the gonads and adrenal after hormonal stimulation was shown by analysis of the data[20] to result from loss of lactogen receptors, rather than a change in the association constant (Table 2). In addition, hormone-induced depletion of lactogenic sites appeared to be cell-specific, since gonadotropin in stimulation had no effect on adrenal or prostatic lactogen binding, and ACTH did not alter the testicular lactogenic sites. As noted above, prolactin receptor loss was not attributable to elevated serum prolactin levels. Recently, a rapid and transient phase of prolactin receptor loss has been described in the mammary gland and liver[21] after treatment with large doses of prolactin, preceding the rise in receptor concentration that results from long-term prolactin treatment[22]. However, the reduction of lactogenic hormone sites we observed after heterologous hormone treatment presumably involves loss of unoccupied binding sites, in contrast to the usual form of receptor down-regulation in which occupancy initiates receptor loss by processing or degradation.

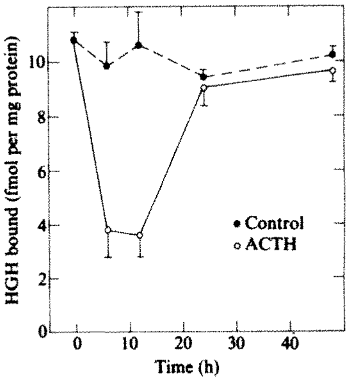

Fig. 3 Rapid loss and recovery of lactogen binding sites in rat adrenal glands after treatment with 10 units of ACTH$_{1-24}$ (○) compared with binding to control adrenals from untreated rats (●). After decapsulation, adrenal glands were homogenised prior to binding analysis with ^{125}I-HGH.

Several explanations can be suggested for these observations on the rapid regulation of lactogenic receptors in steroid-secreting cells. In the gonads, the similar molecular size and coordinate regulation of the LH and lactogen receptors is compatible with a close physical relationship between the two sites. However, while occlusion of the lactogenic sites could be envisaged during occupancy of the gonadotropin receptors, no such cross-reaction has been observed during specificity studies on LH and prolactin receptors. Also, the more rapid return of the lactogenic sites demonstrates their ability to vary independently of LH receptors. It is also conceivable that the loss of prolactin receptors after hormonal stimulation of the gonads and adrenal is part of a more generalised effect on plasma membrane receptor turnover. However, this possiblity is rendered unlikely by the absence of changes in luteal cell receptors for insulin and catecholamines during HCG-induced loss of lactogen receptors.

The receptor loss observed after *in vivo* stimulation of steroidogenesis may be related to an effect of high steroid concentration at the cell membrane, since reduction of adrenal

Table 2 Characterisation of lactogenic binding sites in steroid secreting cells in the basal and stimulated state

Receptor location (treatment)	Equilibrium association constant ($K_a \times 10^9$ M^{-1})*		Binding capacity for HGH (fmol per mg protein)*	
	Controls	Stimulated	Controls	Stimulated
Testis (24 h after oLH—100 μg)	1.3 ± 0.06	0.9 ± 0.06	22.5 ± 0.5	8.8 ± 0.4
Adrenal (6 h after ACTH—1 IU)	1.0 ± 0.15	0.8 ± 0.2	555 ± 45	154 ± 18
Ovary (6 h after HCG—20 μg)	1.2 ± 0.05	1.2 ± 0.06	540 ± 30	243 ± 10

* All values are mean ± s.e.m.

and kidney prolactin receptors follows high dosage treatment with dexamethasone and sex steroids[23]. However, the loss of prolactin receptors in hormone-stimulated gonads and adrenals occurs after the phase of steroid secretion, and is not prevented by treatment with inhibitors of steroidogenesis (unpublished observations). A transient steroid-induced blockade of protein synthesis could also contribute to lactogen receptor loss, since hepatic prolactin receptors undergo transitory loss after cycloheximide treatment[24], and the early actions of ACTH have been shown to include decreased protein synthesis in the adrenal gland[25].

The most likely explanation of lactogen receptor loss is that stimulation of internalisation is a consequence of trophic hormone action on the steroidogenic target cell[26]. Such internalisation of prolactin receptors may be an important consequence of the activation process, particularly if related to the uptake of precursors for steroidogenesis. This mechanism need not be responsible for provision of the cholesterol immediately metabolised during hormone-induced steroid secretion, but would be important for the long-term maintenance of precursor pools within the target cells of the gonads and adrenal. The major substrate for biosynthesis of steroid hormones in the testis, ovary and adrenal glands of the rat is circulating lipoprotein cholesterol, rather than cholesterol that is newly synthesised within the steroid secreting cell[27]. In rats, high density lipoproteins bind to the steroidogenic cell membrane[28] and may be internalised as described for the low density moiety in human cells[29].

A prolactin receptor-mediated mechanism controlling lipoprotein transport would be consistent with the relationship between prolactin and cholesterol metabolism in the gonads[4], and with the high content of prolactin receptors in the ovary, testis, and adrenal gland. Recently, the prolactin molecule has been demonstrated within ovarian luteal cells and adrenal fasciculata cells[30], presumably after receptor binding and internalisation. Furthermore, in the hepatocyte, where lipoprotein synthesis occurs[31], prolactin receptors undergo rapid turnover[24] and a high proportion of the sites are intracellular[32]. In the lactating mammary gland, where lipoproteins are actively metabolised, prolactin receptors are abundant[1] and have been detected within epithelial cells[33]. This evidence for the presence of prolactin receptors within sterol metabolising cells, with the present finding of increased receptor turnover after hormone stimulation, is consistent with the proposal that prolactin receptors could be involved in a mechanism for regulating the entry of lipoprotein precursors in to steroid secreting cells.

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HLA-restricted T-cell recognition of Epstein–Barr virus-infected B cells

A. B. Rickinson, L. E. Wallace & M. A. Epstein

Department of Pathology, Medical School, University of Bristol,
Bristol BS8 1TD, UK

In mice the cytotoxic T-cell response to several types of virus is influenced by genes within the major histocompatibility complex[1–5]; in particular, genetic control is exercised at the effector cell level through a requirement that virus-specific cytotoxic T cells recognise viral antigens in association with H-2K and H-2D region gene products on the surface of infected cells[1,2]. In man the restriction which the analogous HLA-A, -B and -C-region gene products might place on virus-specific T-cell function is still in dispute. The earliest and most controversial evidence concerns the Epstein–Barr virus (EBV), a B lymphotropic agent which causes infectious mononucleosis[6] (IM) and which induces an unusually vigorous T-cell response[7]; cytotoxic T cells from IM patients' blood were shown to be EBV-specific, yet, in contrast to mouse systems, apparently free of any obvious HLA restriction[8,9]. Since then T-cell recognition of EBV-infected B cells has assumed particular significance as a model system for the study of cytotoxic T-cell function in man. This report describes the results of a new approach clearly indicating that HLA-A and -B region products do indeed have a role in this system.

During recent experiments monitoring the ease of establishment of EBV-transformed B-cell lines from cultures of adult donor leukocytes exposed to the virus *in vitro*, it was found that cultures from non-infected (seronegative) individuals regularly gave rise to cell lines, whereas in the corresponding cultures from previously-infected (seropositive) donors the early proliferation of virus-infected B cells was halted by a T-cell dependent mechanism[10]. All the available evidence suggested that this regression of growth was occurring through the reactivation *in vitro* of a pool of EBV-specific cytotoxic T-cell precursors present exlusively in the blood of seropositive individuals[11,12]. In

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the experiments reported here T cells collected from regressing cultures have been tested for their ability to inhibit the outgrowth of EBV-transformed B cells of the autologous line and of HLA-related and unrelated allogeneic lines.

Ten healthy adult donors of known HLA type and of known serological status with respect to EBV were used. Experimental procedures were as previously described[10–12]. From each 60-ml blood sample, 7×10^6 mononuclear cells were used in a microtest plate assay for which the cells were exposed to EBV, or to culture medium as a control, before seeding in 0.15-ml volumes into microtest plate wells at one of a range of concentrations (2×10^6 to 1.25×10^5 cells per ml); the cultures were then observed for 28 d to assess the 'strength' of regression for each donor in terms of the minimum initial concentration required for a 50% incidence of the effect among replicate cultures[10]. The remaining mononuclear cells were exposed to EBV, cultured at 2×10^6 per 2-ml well and then 10–14 days later the cultures were collected and the T-cell subpopulation prepared. Aliquots of 10^5 T cells were then added to microtest plate wells containing known numbers (ranging from 2×10^5 to 2.5×10^2) of autologous or of allogeneic EBV-transformed target cells of known HLA type; three to five replicate wells were set up for each particular combination, along with parallel cultures of target cells alone. After 2 weeks the incidence of successful outgrowth of the target cells could be scored unequivocally[12] and, for each particular effector and target cell combination, the growth inhibitory effect of the T cells was expressed as the ratio of that number of target cells required for a 50% incidence of successful outgrowth in the presence of T cells, to that number required in cultures of target cells alone.

Table 1 summarises the data from all 10 donors studied. The results clearly emphasise the immunological basis of regression in that the effect was consistently observed in cultures set up from all seven seropositive donors tested, with individual donors tending to show either strong (CA, SG, BW) or somewhat weaker (MM, AR) responses on repeated testing, but was never seen in cultures from the seronegative donors. This pattern of results was exactly reflected in the co-cultivation assay where T cells collected from seropositive donor cultures just before their regression consistently showed a marked inhibition of outgrowth of the autologous EBV-transformed cell line, whereas very little growth inhibition was apparent in the corresponding co-cultures from three seronegative donors.

Allogeneic cell lines used as targets in the co-cultivation assay were likewise hardly affected by the addition of T cells from seronegative donor cultures; in contrast effector cells from seropositive donor cultures did in some cases cause significant inhibition, although this was almost invariably less than that mediated by these same cells against their autologous cell line. Using the degree of autologous target cell inhibition within each experiment as a standard, the relative inhibition suffered by allogeneic cell lines was analysed in the light of their known HLA-A, -B and -C antigen expression. This approach revealed an underlying pattern in the results which is illustrated by the representative data shown in Fig. 1 from three seropositive donors, CA, MM and SG. Whilst an analysis in relation to HLA-C antigen expression is difficult in view of the incomplete typing at this locus, there are strong indications that sharing of HLA-A or -B antigens between effector and target cells predisposes towards significant cross-reactivity. Thus inhibition of

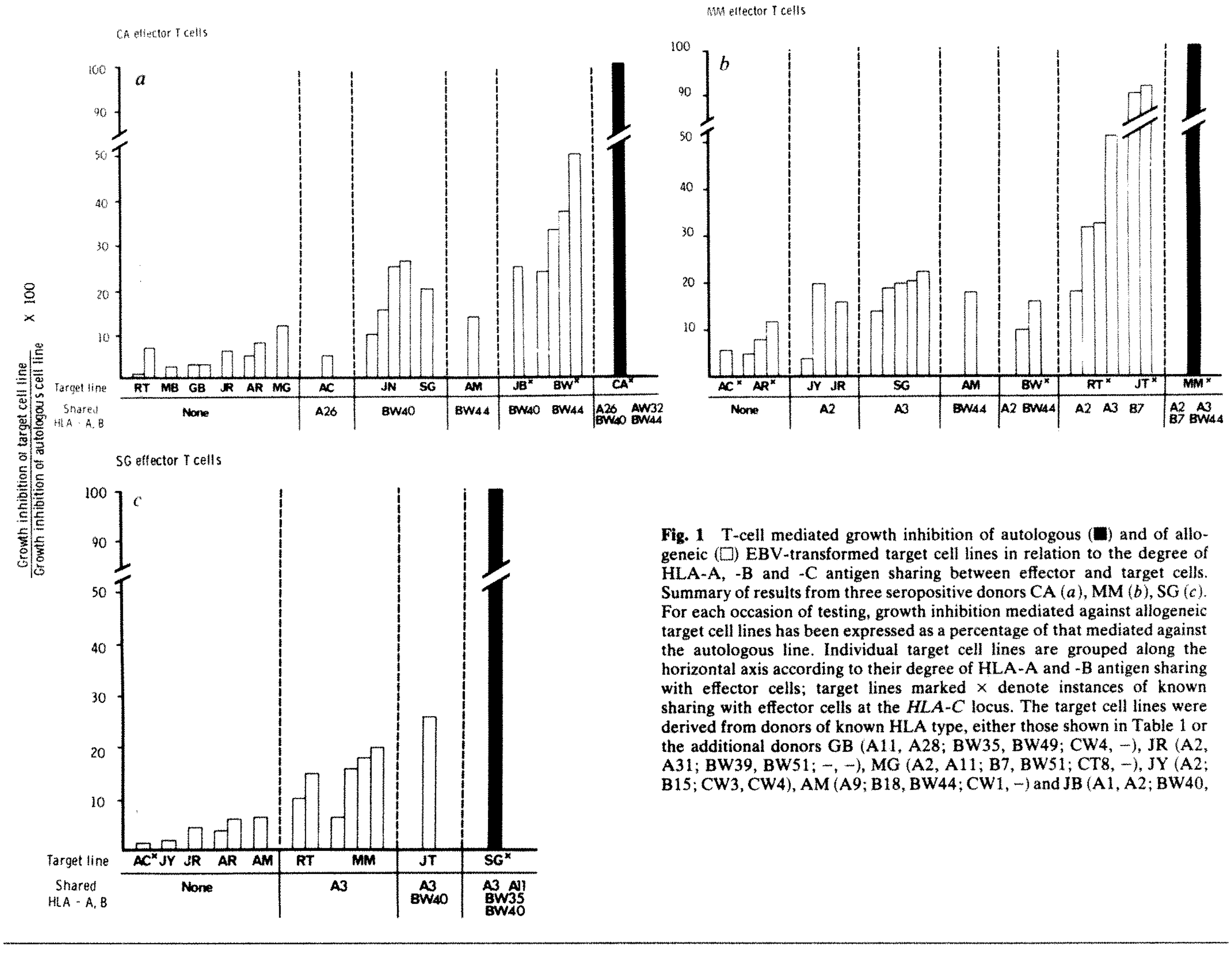

Fig. 1 T-cell mediated growth inhibition of autologous (■) and of allogeneic (□) EBV-transformed target cell lines in relation to the degree of HLA-A, -B and -C antigen sharing between effector and target cells. Summary of results from three seropositive donors CA (*a*), MM (*b*), SG (*c*). For each occasion of testing, growth inhibition mediated against allogeneic target cell lines has been expressed as a percentage of that mediated against the autologous line. Individual target cell lines are grouped along the horizontal axis according to their degree of HLA-A and -B antigen sharing with effector cells; target lines marked × denote instances of known sharing with effector cells at the *HLA-C* locus. The target cell lines were derived from donors of known HLA type, either those shown in Table 1 or the additional donors GB (A11, A28; BW35, BW49; CW4, –), JR (A2, A31; BW39, BW51; –, –), MG (A2, A11; B7, BW51; CT8, –), JY (A2; B15; CW3, CW4), AM (A9; B18, BW44; CW1, –) and JB (A1, A2; BW40,

Table 1 EBV-specific effector T-cell assays for donors of known HLA type and antibody status with respect to EBV

Donor* (Antibody status to EBV)	HLA type†			Effector T-cell assays		
				No. of experiments	Cell concentrations for 50% regression‡	Growth inhibitions of autologous cell line§
CA (+)	A26 AW32;	BW40 BW44;	CW3 CW5	4	4.8×10^5 cells ml^{-1}	170
MM (+)	A2 A3;	B7 BW44;	CW5 CT8	6	7.3×10^5 cells ml^{-1}	38
SG (+)	A3 A11;	BW35 BW40;	CW2 CW4	4	3.3×10^5 cells ml^{-1}	120
BW (+)	A2;	BW40 BW44;	CW3 CW5	5	3.2×10^5 cells ml^{-1}	108
JN (+)	AW24 AW33;	B8 BW40;	— —	4	8.3×10^5 cells ml^{-1}	79
AR (+)	A1;	B8 B17.1;	CW6 CT8	6	7.5×10^5 cells ml^{-1}	15
JT (+)	A2 A3;	B7 BW40;	CW6 CT8	1	8.0×10^5 cells ml^{-1}	20
RT (−)	A2 A3;	B7 B15;	CT8 —	3	$>2.0\times10^6$ cells ml^{-1}	2
AC (−)	A1 AW26;	B8 BW45;	CW6 CT8	2	$>2.0\times10^6$ cells ml^{-1}	2
MB (−)	A1 A3;	B8 B14.2	— —	1	$>2.0\times10^6$ cells ml^{-1}	3

* Antibody status with respect to EBV was determined by the anti-virus capsid antigen immunofluorescence test as described elsewhere[10].

† Typing at the HLA-A, -B and -C loci was carried out on freshly isolated blood mononuclear cells at the National Tissue Typing Reference Laboratory. Undefined *C* locus antigens are shown as blanks.

‡ The minimum initial cell concentration for a 50% incidence of regression was calculated from the incidence of transformation and/or regression seen in replicate cultures in the 4-week microtest plate assay[10]. Mononuclear cells were exposed either to culture medium alone or to an EBV preparation (10^5 50% transforming doses per ml) from B95-8 culture supernatant before seeding in 0.15-ml volumes into microtest plate wells (Nunc) at 2×10^6, 10^6, 5×10^5, 2.5×10^5 and 1.25×10^5 cells per ml and culturing for 4 weeks. The figure given for each donor is the mean of those obtained in the number of experiments shown. A value of $>2\times10^6$ cells per ml indicates that regression was not seen in any of the cultures.

§ EBV-infected mononuclear cell cultures in 2-ml wells (Linbro) were collected 10–14 d post infection and dead cells removed by a preliminary centrifugation for 30 min at 400*g* on Ficoll-Hypaque. The T-cell subpopulation was then prepared by rosetting with AET-treated sheep erythrocytes according to the method of Gmelig–Meyling and Ballieux[22] and the rosetted cells rescued by lysis of the erythrocytes in 0.75% NH_4Cl in 0.016M Tris buffer, *p*H 7.25, at 4 °C. Aliquots of 10^5 such T cells were added to replicate microtest plate wells containing known numbers (2×10^5, 10^5, 5×10^4, 2.5×10^4, 10^4, 5×10^3, 2.5×10^3, 10^3, 5×10^2 and 2.5×10^2) of target cells of the autologous EBV-transformed B-cell line and the incidence of successful target cell outgrowth was recorded after 2 weeks[12]. T-cell mediated growth inhibition was expressed as the ratio of that number of target cells required for a 50% incidence of successful outgrowth in the presence of T cells to that number required for successful outgrowth in cultures of target cells alone. The figure given for each donor is the mean of those obtained in the number of experiments shown.

growth over and above that seen against HLA-unrelated targets was consistently found in some, though not all, examples of a single shared antigen (for example, reciprocal recognition of MM and SG through shared A3, Fig. 1*b*, *c*), whereas more extensive antigen sharing was almost always associated with an enhanced cross-reactivity. For instance, the recognition of BW targets by CA effectors through shared BW40, BW44 (Fig. 1*a*) and of JT targets both by SG effectors through shared A3, BW40 (Fig. 1*c*) and by MM effectors through A2, A3, B7 (Fig. 1*b*) was in each case confirmed by the reciprocal target and effector cell combination (data not shown).

It seems most unlikely that results of this type could have arisen by chance or could be explained on the basis of differences in the essential sensitivity to growth inhibition *per se*. Thus, whilst individual results from such a small sample of randomly selected donors must be interpreted cautiously, the overall data are consistent with the concept of an HLA-related T-cell cytotoxicity similar to that recently reported for influenza virus-specific human T cells, again studied after secondary stimulation *in vitro*[13]. Furthermore, the apparent relationship between the magnitude of effector cell function and the extent of HLA antigen sharing with target cells suggests that the effector cell population contains a number of independent clones, each recognising the target antigen in association with one particular HLA region gene product. Within this framework there are individual examples of poor recognition despite HLA antigen sharing (for example, CA effectors and AC targets sharing A26, Fig. 1*a*) and such exceptions, perhaps related to those now being recognised in the influenza virus system[14–16], require further investigation.

The rapid inhibition of growth caused by effector T cells in the co-cultivation assay[12] is almost certainly a consequence of their direct cytotoxic action. Thus chromium release assays carried out in this[17] and in another[18] laboratory have now shown that effector T cells from regressing cultures are both cytotoxic and EBV-specific, as they kill autologous EBV-transformed cells but not autologous pokeweed mitogen-stimulated B lymphoblasts or cells of the K562 cell line, a sensitive indicator of nonspecific natural killer cell activity[19]. If, as seems likely, these reactivated T cells recognise the same EBV-associated cell surface change as do primary effector T cells present in the blood of acute IM patients (that is, the lymphocyte-detected membrane antigen, LYDMA[8]) it becomes important to re-examine the possibility of HLA-related constraints governing IM T-cell cytotoxicity[20,21]. Certainly this work and other independent studies[18] strongly suggest that cytotoxic T cells reactivated *in vitro* do indeed recognise an EBV-associated LYDMA-like antigen in conjunction with HLA-A and -B region gene products on the surface of infected B cells. This conclusion is strengthened by our most recent experiments demonstrating that such recognition can be specifically blocked by pretreatment of the target cells with high titre preparations of monoclonal antibodies to a common determinant on HLA-Ag, -B and -C molecules.

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Hyperpolarisation of rat peritoneal macrophages phagocytosing latex particles

J. Kouri, M. Noa, B. Diaz & E. Niubo

Centro Nacional de Investigaciones Cientificas, Havana, Cuba

The changes that take place in the plasma membrane during endocytosis are still poorly understood[1]. Studies of the electrical properties and related permeabilities of cells during pinocytosis and phagocytosis should provide insight into these changes. This approach has not been much exploited, although there have been a few relevant reports. For example, induction of pinocytosis in amoebae produces a marked decrease in plasma membrane resistance[2], and exposure of polymorphonuclear leukocytes to phagocytosable particles can engender alterations in ionic permeabilities[3]. The present report demonstrates that the induction of phagocytosis in rat peritoneal macrophages is accompanied by a sustained hyperpolarisation of the cells.

Macrophages were obtained by the implantation of agar-coated sterile Teflon disks in the peritoneal cavities of Wistar rats[4]. After 3 d the disks were removed and placed in Tyrode solution (NaCl, 124 mM; KCl, 3 mM; $CaCl_2$, 2.5 mM; $MgCl_2$, 1.2 mM; $NaHCO_3$, 15 mM; NaH_4PO_4, 1.2 mM; glucose, 5 mM, *p*H 7.4, kept at 37 °C with 95% O_2–5% CO_2 bubbling through). The disks were placed in an electrophysiological chamber and macrophages were selected with an inverted phase microscope. The cells were penetrated with glass microelectrodes of less than 0.5 μm diameter filled with 3 mM KCl. Electrode resistances were in the range of 20–50 MΩ and tip potentials were less than 5 mV. The microelectrodes were coupled through a high impedence compensator to a differential preamplifier MZ4 (Nihon–Khoden) and a VC-8 oscilloscope (Nihon-Khoden). Records were photographed with a cine camera running at velocities of 10 or 20 mm s^{-1}.

As in previous work by our laboratory[5–7], most of the cells penetrated showed distinct, negative potentials (Fig. 1*a*) which disappeared when the electrodes were withdrawn. For the 200 cells studied in the present work, the mean potential was −13 ± 4.7 (s.d.) mV. The potentials were stable for more than 30 min, but disappeared on addition of 2 mM NaF or 2.5 mM DNP. They were similar to the potentials we have obtained previously, and to those reported for macrophages of other species[8,9] and for other non-excitable cells[10–14]. Occasionally cells in our preparations showed positive potentials whose nature is under study and will not be discussed here.

Difco latex (0.8 μm diameter spheres, washed and diluted 1:10 in phosphate-buffered saline: NaCl, 137 mM, KCl, 2.7 mM, buffered at *p*H 7.2–7.4 with 10 mM sodium phosphate) was added to the disks in the chamber (0.06 ml of latex added to 20 ml of Tyrode). Latex induces extensive phagocytosis which persists for at least 20–30 min[15]. During this time, approximately 10 particles per cell are taken up. Latex was added to impaled cells 2–3 min after they had been penetrated. When this was done there was a rapid hyperpolarisation (Fig. 1*b*) followed by a gradual hyperpolarisation which reached a maximum at 15–30 min (Fig. 1*c*). The mean initial potential measured in the first minute after exposure to latex was −25 ± 3.2 and the eventual hyperpolarisation was to −46 ± 5.1 mV (40 cells). After 20–30 min, the membrane depolarised towards the prestimulation level (Fig. 1*d*). In 80% of the cells responding to latex we have observed short trains (< 1 s) of small (up to 5 mV) brief depolarisations and hyperpolarisations or slower oscillations of up to 2 mV and 3 s duration, superimposed on the longer term hyperpolarisation. Addition of phosphate-buffered saline without latex or of a drop of Tyrode solution does not provoke changes in the membrane potential.

Gallin and coworkers[8,9] working on macrophages of other species have reported transient hyperpolarisations occurring spontaneously and in response to chemotactic factor isolated from serum. They have suggested that the hyperpolarisations might reflect increased permeability to potassium, perhaps through events in which calcium is involved. When we increased the concentration of calcium in our medium to 3.8 mM we observed an increase in the mean potential measured in unstimulated cells to −20 ± 2.2 mV (30 cells). With such cells latex evokes hyperpolarisation to −50 ± 4.3 mV (10 cells).

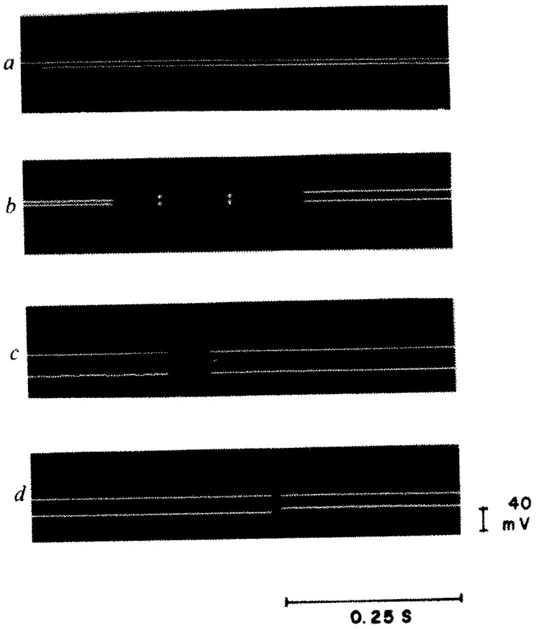

Fig. 1 Records obtained from the same cell at different stages in the response to latex. *a*, Entry of the electrode into the cell. *b*, Addition of latex. The traces on the left show the potential just before the addition of latex. The camera was then stopped, advanced two frames (producing the two pairs of dots to mark the time of addition of latex), latex was added and recording resumed 30 s later. The traces on the right thus show the potential 30 s after adding latex. A distinct hyperpolarisation has occurred. *c*, Potentials 15 (left) and 20 (right) min after adding latex. The camera was stopped between the two recording periods producing the gap in the record. Note the hyperpolarisation. *d*, Potentials 25 (left) and 30 (right) min after adding latex. The camera was stopped between the two recording periods producing the gap in the record. Note the decrease of the potential.

The present investigation and the studies cited above suggest that the early events in the induction of endocytosis include changes in the cell's permeability to ions. For granulocytes, it is reported that alterations in membrane potential can be induced by interaction of molecules that stimulate endocytosis with the cell surface; the potential changes do not require actual ingestion of the molecules[16], but whether this is generally the case is not known. It is too early for us to speculate in detail on the significance of the changes we observe or on how the permeability changes are related to the various stages of endocytosis or to the properties of the cell-surface receptors involved (presumably in our case, relatively nonspecific receptors[1] are involved). However, given the many parts evidently played by ions such as Ca^{2+}, K^+ and so forth in controlling the distribution or functional state of such cellular elements as contractile proteins, there are many possibilities for their participation in influencing endocytic processes. In any event, the work reported

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here suggests that electrophysiological techniques will prove useful in future analyses of cell-surface changes related to endocytosis by mammalian cells.

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Electrophysiological responses of *Didinium nasutum* to *Paramecium* capture and mechanical stimulation

Ritsuo Hara & Hiroshi Asai

Department of Physics, Waseda University, Okubo, Shinjuku-ku, Tokyo 160, Japan

***Didinium nasutum* is a carnivorous ciliate with a preference for feeding on species of *Paramecium*. Accidental contact between a *Paramecium* and the proboscis of a hungry *Didinium* (Fig. 1*a*) causes instantaneous discharge of extrusive organelles from the proboscis. If the strike is successful, some of these organelles penetrate the *Paramecium*, which immediately stops swimming and discharges many trichocysts, mostly from the struck side (Fig. 1*b*). Cytoplasmic streaming pulls the discharged organelles inward, the captured *Paramecium* reaches the proboscis, and then the *Didinium* swallows the prey, opening its proboscis[1–10] (Fig. 1*c* and *d*). On the other hand, a hungry *Didinium* does not discharge extrusive organelles when it collides with a glass wall. Instead it shows an avoiding reaction. When a *Paramecium* collides with the proboscis of a fed *Didinium*, the proboscis similarly does not discharge extrusive organelles, and the fed *Didinium* makes an avoiding response. We found that a hungry *Didinium* held steady by two glass capillary microneedles performed normal capture and ingestion in a solution containing *Paramecium caudatum*. We describe here an electrophysiological investigation of *Didinium nasutum* in collision with *P. caudatum* or with a glass rod while held still by glass capillary microneedles.**

Methods of stimulation and recording were similar to those used by Naitoh and Eckert for electrophysiological investigations of *Paramecium*[11]. Typical results are shown in Fig. 2. Accidental contact between a *Paramecium* and the proboscis of a fixed hungry *Didinium* generated a transient hyperpolarising potential of the *Didinium* cell, followed by a depolarising action potential associated with discharge of the extrusive organelles (Fig. 2*a* and *b* and Fig. 2*c* and d_2). The peak of the depolarising potential corresponded to completion of the discharge of the extrusive organelles. Subsequently, the membrane potential returned slowly to resting level. Marked fluctuations of the membrane potential often occurred during this period (Fig. 2*c* and d_2). Weak reversals of ciliary movement were associated with depolarisations of small magnitude. On rare occasions after discharging extrusive organelles, *Didinium* failed to capture the target organism, possibly because of a poor hit. *Didinium* then simply withdrew the discharged extrusive organelles. Even in this case, the changes in membrane potential were similar to those occurring in cases of successful capture.

Mechanical stimulation of the apex of the proboscis of a hungry *Didinium* with a glass rod induced a transient depolarising mechanoreceptor potential, which was followed by a depolarising action potential of the general membrane (Fig. 2*f* and *g*). No discharge of extrusive organelles was observed, but the *Didinium* showed ciliary reversal in response to this stimulation. A spontaneous action potential was often recorded in *Didinium* cells (Fig. 2d_1), and this also caused ciliary reversal.

In some cases, the proboscis of a fixed fed *Didinium* happened to collide with a new *Paramecium* about 5 min after ingestion of the previous prey. In such cases, the membrane responses were the same as for mechanical stimulation of the proboscis of a fixed hungry *Didinium* (Fig. 2*e*). Fed *Didinium* never discharged extrusive organelles, but responded to contact with *Paramecium* by ciliary reversal, in the same way as to a glass rod.

The proboscis membrane of a hungry *Didinium* seems to have a chemoreceptor that responds to contact with *Paramecium*, as well as a mechanoreceptor able to sense external mechanical stimulation. Chemoreception induces a hyperpolarising potential, and mechanoreception induces a depolarising potential. On the other hand, only the mechanosensitive system of the proboscis membrane is active in a fed *Didinium*. According to Naitoh and Eckert, mechanical stimulation of the anterior surface of *Paramecium* induces a mechanosensitive depolarising potential. In wild type cells a regenerative calcium action potential causing ciliary reversal is always accompanied by the anterior mechanoreceptor potential, caused by an increase in Ca^{2+} permeability after mechanical stimulation[12,13]. The responses of the proboscis surface of hungry *Didinium* to mechanical stimulation and that of the proboscis of fed *Didinium* to collision with a *Paramecium* are similar to the above responses of *Paramecium*.

The *Paramecium*-sensitive hyperpolarising potential and the depolarising action potential associated with discharge of the extrusive organelles were characteristic features of the prey capture behaviour of *Didinium*. The action potential was always

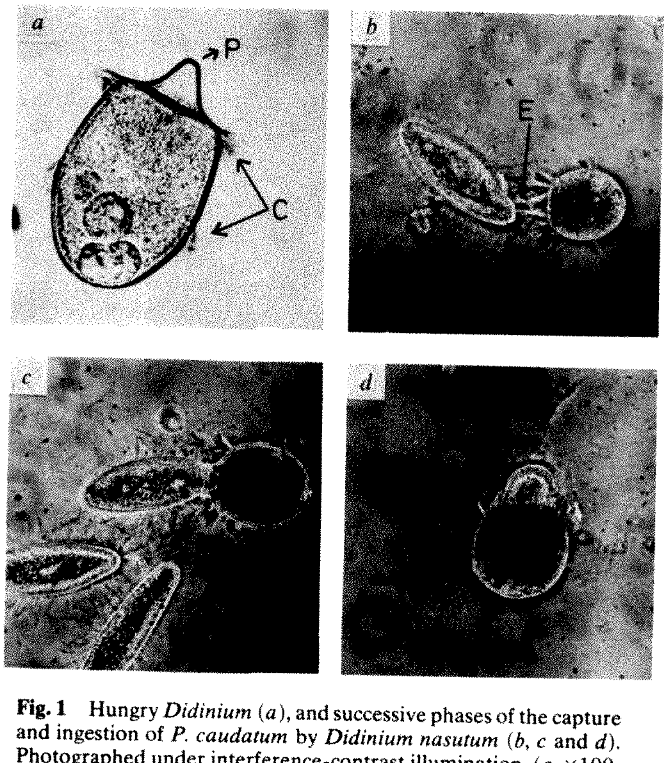

Fig. 1 Hungry *Didinium* (*a*), and successive phases of the capture and ingestion of *P. caudatum* by *Didinium nasutum* (*b*, *c* and *d*). Photographed under interference-contrast illumination. (*a*, ×100; *b*, *c* and *d*, ×50.) P, proboscis; C, cilia; EO, extrusive organelles.

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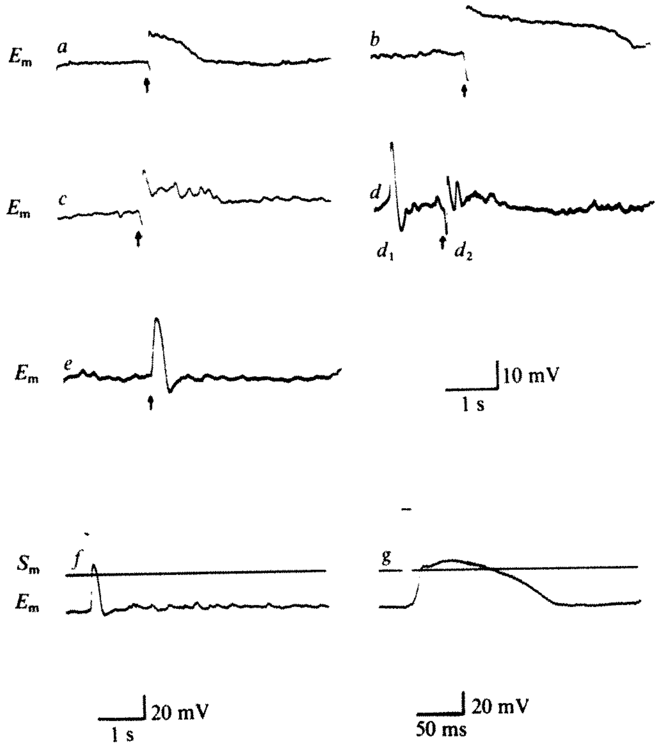

Fig. 2 Responses of the membrane potential to collision between *P. caudatum* and the apex of the proboscis of *Didinium nasutum* (*a*, *b*, *c*, d_2 and *e*). Arrows show the moment at which a *Paramecium* collided with the apex of the proboscis of *Didinium*. Spontaneous ciliary reversal response is also shown (d_1), together with responses to mechanical stimulation of the apex of the proboscis (*f* and *g*). *a*, *b*, *c*, *d*, *f* and *g*, hungry *Didinium*; *e*, fed *Didinium*. E_m, membrane potential; upward and downward deflections correspond to depolarisation and hyperpolarisation of the membrane, respectively. S_m, deflection in the trace indicates the duration and relative intensity of the electric pulse activating a piezoelectric crystal to drive a glass rod against the surface of the membrane. Membrane potentials were recorded in mixtures of 1 mM K^+, 1mM Ca^{2+} and 1 mM Tris–maleate (*p*H 7.0) (*a*, *b*, *c*, *d* and *e*) and 1 mM K^+, 1 mM Ca^{2+} and 1 mM Tris-HCl (*p*H 7.2) (*f* and *g*). All measurements were made at room temperature (20–25° C).

accompanied by the hyperpolarising potential. It seems likely that the discharge of extrusive organelles is associated with an inflow of Ca^{2+} through the proboscis membrane due to a sudden increase in Ca^{2+} permeability, enabling Ca^{2+} to flow inwards down its electrochemical gradient. This is because the discharge of extrusive organelles was always associated with the depolarising action potential, which was different from a general membrane depolarising action potential causing ciliary reversal, and membrane depolarisation in this species seems to involve an inflow of Ca^{2+}, as in the case of *Paramecium* in a mixture of 1 mM K^+ and 1 mM Ca^{2+}. It has been shown that the discharge of *Paramecium* trichocysts is associated with a flow of Ca^{2+} through the membrane[14].

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Estimation of cytoplasmic and vacuolar *p*H in higher plant cells by ^{31}P NMR

Justin K. M. Roberts*, Peter M. Ray*,
Norma Wade-Jardetzky† & Oleg Jardetzky†

* Department of Biological Sciences, Stanford University, Stanford, California 94305
† Stanford Magnetic Resonance Laboratory, Stanford University, Stanford, California 94305

It has recently been emphasised that the intracellular *p*H of plant cells must be actively regulated to offset acid–base imbalances that occur during metabolism and solute uptake (for review see ref. 1). Changes in cytoplasmic *p*H might be important (1) in controlling various aspects of metabolism[2], including enzyme activities responsible for malate synthesis during cation accumulation[1,2], (2) in polar transport of the plant hormone, auxin[3], and (3) in intracellular responses to substances such as auxin and the phytotoxin fusicoccin, which cause proton extrusion[1,4,5]. Almost no direct measurements of cytoplasmic *p*H have been made on the small cells typical of higher plants[1]. The task is hampered by the presence of a generally acidic vacuole which occupies much of the cell volume. Use of the indirect method of ^{14}C-5, 5-dimethyloxazolidine-2, 4-dione (DMO) distribution is subject to considerable uncertainties because of corrections needed for vacuolar *p*H and volume, and DMO metabolism[6,7]. Here we report the use of ^{31}P nuclear magnetic resonance (NMR) to estimate the *p*H of vacuole and cytoplasm in maize root tips.

The observation of intracellular organic and inorganic phosphates by ^{31}P NMR has been proposed as a method for determining the internal *p*H of animal and microbial cells[8,9]. As the chemical shift (δ, the resonance frequency relative to a standard) of ^{31}P in these compounds yields a titration curve when plotted against *p*H (insert in Fig. 1), a determination of *p*H from a measured δ value is possible in principle. The advantage of the method is that it is noninvasive and nondestructive. The principal problem in its use is that *p*H may be the dominant, but is not the sole determinant, of δ. However, the resulting ambiguities are not severe, as will be discussed below.

Figure 1*a* shows a typical ^{31}P-NMR spectrum of maize root tips showing resonances from glucose-6-phosphate (G-6-P, peak 1) and inorganic phosphate (P_i, peaks 2 and 3). The

Table 1 ^{31}P-Chemical shifts observed in maize root tips, and corresponding *p*H values

	δ (p.p.m.) of peak relative to MDP			Corresponding *p*H values		
Treatment	1	2	3	1	2	3
Control	12.6	14.7	16.4	7.25	7.15	5.5
Control	12.65	14.7	16.4	7.1	7.15	5.5
Control	12.6	14.75	16.4	7.25	7.05	5.5
2-dG*	12.68	—	16.45	7.2	—	5.4
6 mM NH_4OH†	12.58	14.6	16.0	7.3	7.2	6.05
12 mM NH_4OH†	12.55	14.55	15.6	7.4	7.3	6.4

Control root tips were prepared and treated as for Fig. 1*a*. Columns headed 1, 2 and 3 refer to NMR peaks 1, 2 and 3 illustrated in the Figs 1 and 2.

* Root tips were incubated for 2 h in 50 mM 2-dG at 23 °C. *p*H value given for peak 1 is based on the titration curve of 2-dG-6-phosphate. Peak 2 was too small to be assigned a δ value in 2-dG-treated tissue.

† Root tips were incubated in 10 ml of 6 or 12 mM NH_4OH (initial *p*H 9.6) at 23 °C for 30 min (final *p*H 6.56 and 7.2, respectively).

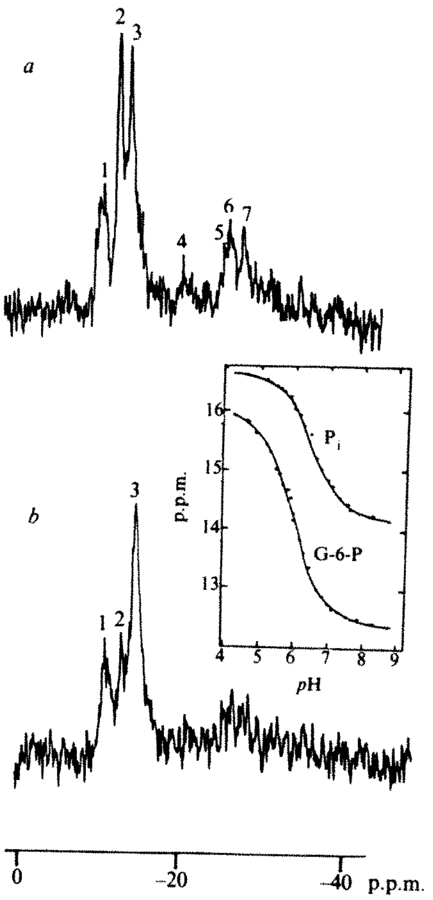

Fig. 1 *a*, 145.7 MHz ^{31}P-NMR spectrum of maize root tips (~900 tips or ~1.2 g) 1.5 mm long. Maize (*Zea mays* L.) WF9 × Bear 38 were soaked for 6 h in tap water, and grown for 2 days on moist tissue in the dark, at 25 °C. Plants were collected in the light, root tips 2–3 mm long being excised with a razor blade, and then put on ice. Before being placed in the NMR tube, they were washed three times with ice-cold distilled water. (Washing with 1 mM EDTA did not affect the quality of the spectra obtained.) Spectra were obtained at 5 °C with ~400 scans at 0.409 s per scan without proton irradiation and without lock using a modified Bruker HXS-360 spectrometer. Shifts are referenced to 0.05 M methylene diphosphonic acid (MDP) in *p*H 8.9 Tris buffer, located in a coaxial capillary tube. *b*, Spectrum of root tips 2.5 mm long, grown and prepared as *a*. Peak 1, G-6-P; 2, cytoplasmic P_i; 3, vacuolar P_i; 4 and 5, ATP; 6 and 7, UDP-Glc.

individual peaks can be identified on the basis of the relative chemical shifts and the published analyses of phosphate compounds in growing plant tissues[10–13]. Enzymatic analysis[14] of maize root tip perchloric extracts confirms that G-6-P is the predominant hexose phosphate.

The resonance due to G-6-P in maize root tips has a δ value corresponding to *p*H 7.1–7.25 (Table 1). Because G-6-P is an important cytoplasmic metabolite, and is not considered to form a significant storage pool, the G-6-P resonance probably reflects the *p*H of the cytoplasm. The positions of the two clearly resolvable peaks in the P_i region of the spectrum correspond to P_i at *p*H 7.05–7.15 (peak 2) and *p*H 5.5 (peak 3). This suggests that we are detecting resonances from two separate compartments in these cells—cytoplasm (*p*H ~ 7.1) and vacuole (*p*H ~ 5.5) These inferences are suported by the following observations:

(1) A spectrum (Fig. 1*b*) obtained from longer root segments, which contain much more highly vacuolate cells, shows much smaller peaks 1 (G-6-P) and 2 (cytoplasmic P_i), relative to peak 3 (vacuolar P_i), than are seen in root tips (Fig. 1*a*), the cells of which contain relatively more cytoplasm. In the more vacuolate tissue (Fig. 1*b*) the peaks corresponding to other important ^{31}P-containing cytoplasmic metabolites (ATP, UDP-Glc) are also reduced. A highly vacuolate tissue, such as the coleoptile of maize, yields only one ^{31}P resonance, corresponding to P_i at *p*H 5.5, that is, peak 3 (data not shown).

(2) When a root tip sample is aged at ~5 °C for 2–3 h in a closed (and thus anaerobic) NMR tube, peak 1 (G-6-P) decreases and peak 2 (cytoplasmic P_i) increases correspondingly, without much change in peak 3 (vacuolar P_i) (Fig. 2*a*, *b*). This presumably reflects depletion of G-6-P, as is known to occur in conditions of oxygen starvation[10,11], with release of P_i but without loss of the normal compartmentation of the cells. When tissue is similarly aged for 7–8 h the G-6-P peak disappears entirely, and the two P_i peaks (2 and 3) merge into a single peak that has a δ value intermediate between the two (not shown). This is to be expected as the cell die and their internal compartmentation breaks down.

(3) If root tips are incubated with 2-deoxyglucose (2-dG), a large ^{31}P resonance in the sugar–phosphate region appears (Fig. 2*c*). This peak can be attributed to 2-dG-6-phosphate, the principal metabolite formed when cells take up 2-dG[15,16]. Concomitantly, peak 2 (cytoplasmic P_i) virtually disappears, whereas peak 3 (vaculor P_i) remains strong. This corresponds with the expectation that cytoplasmic P_i will be used to phosphorylate 2-dG in the cytoplasm, where hexokinase is located.

(4) When root tips are incubated in dilute solutions of the permeant base, ammonia, the δ values of peaks 1 and 2 show little change, whereas that of peak 3 changes substantially,

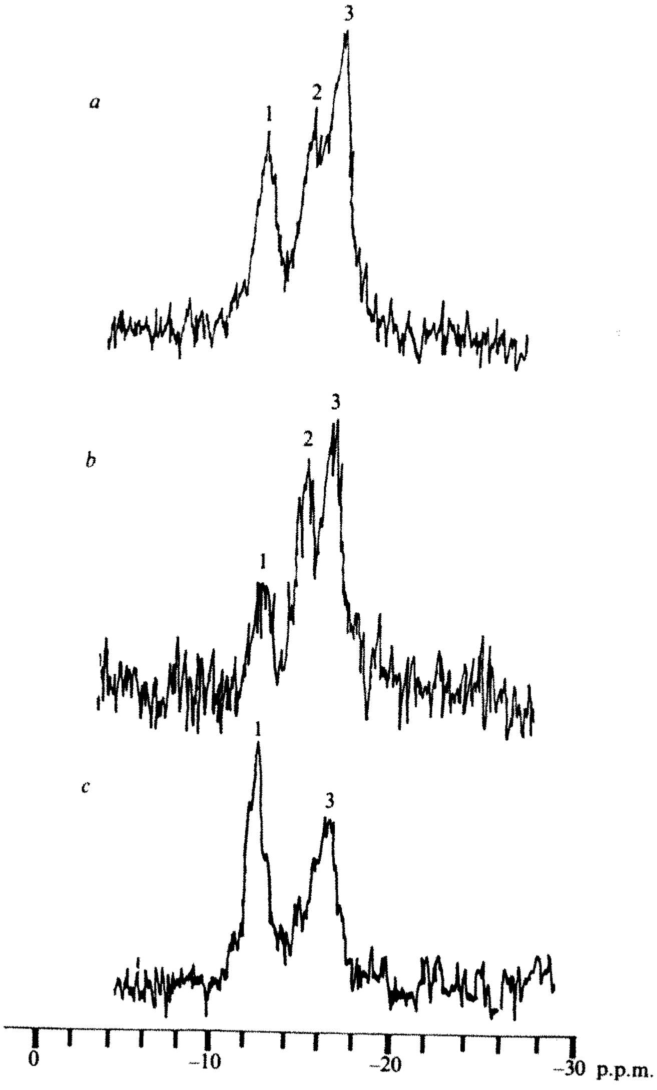

Fig. 2 ^{31}P-NMR spectra obtained from: *a*, maize root tips prepared as in Fig. 1*a*. *b*, The same root tips sample as in *a* after 2 h at 0–5 °C in the NMR tube; peaks 1, 2 and 3 as in Fig. 1*a*. *c*, Root tips that had been incubated in 50 mM 2-dG for 2 h at 23 °C; peaks 1, 2-dg-6-phosphate; 3, vacuolar P_i.

corresponding to a substantial rise in pH (Table 1). This suggests again that peaks 1 and 2 are due to ^{31}P in one compartment, peak 3 to ^{31}P in a different compartment. These results also indicate, as might be expected[1], that the cytoplasmic pH of these cells is regulated much more closely than that of the vacuole.

The pH values reported here should be regarded as approximate because the relationship between the δ values of ^{31}P compounds, and the pH as measured with a glass electrode, can differ in media of different composition. Although it has been reported[9] that Mg^{2+} has no effect on the titration curve of P_i, we find that both Mg^{2+} and concentrated protein shift the apparent pK_a of P_i and G-6-P to a lower value, by as much as 0.2–0.4 pH units. These effects will be described in detail elsewhere. The difference in apparent pH between the two compartments detectable by NMR in this tissue is, however, much larger than could be caused by known perturbations of the ^{31}P compounds. The ^{31}P method can therefore be useful for testing whether intracellular pH changes during certain physiological responses[1].

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Canavanine potentiates the response of insect malpighian tubules to diuretic hormone and cyclic AMP

Ada Rafaeli & Shalom W. Applebaum

Department of Entomology, Faculty of Agriculture,
The Hebrew University, Rehovot, Israel

Insects regulate the amount of water they excrete via their malpighian tubules, the degree of stringency depending on the hydration of their diet and on the frequency at which they feed. The excretion of water is controlled by a diuretic hormone, which is released as required into the haemolymph by the corpora cardiaca[1,2]. Previous reports[3–5] have shown that non-protein amino acids, known to be neurotoxic in vertebrates[6] as well as in insects[7], lead to a disruption of water balance in insects. However, the exact mode of action of these amino acids has not been elucidated. We report here on preliminary experiments using isolated malpighian tubules of locusts (*Locusta migratoria migratorioides* (R & F)) which show that the non-protein amino acid L-canavanine inhibits fluid secretion *in vitro* by isolated tubules. Furthermore, when tubules, previously inhibited by canavanine, are subsequently stimulated by exogenous diuretic hormone (methanol extracts of storage lobes of corpora cardiaca[1,2]) or cyclic AMP (which mimics the action of diuretic hormone[1]), the stimulation obtained is much greater than that normally expected from diuretic hormone or cyclic AMP.

Table 1 The enhanced effect of cyclic AMP and diuretic hormone stimulation after L-canavanine treatment

Response to:	% Increase in rate of fluid secretion	
	s Control tubule	Canavanine-treated tubules
Cyclic AMP (10^{-3} M)	61.79 ± 5.90 (n = 13)	764.18 ± 45.81 (n = 11)
Diuretic hormone (5 pairs storage lobes)	88.83 ± 8.41 (n = 10)	339.99 ± 50.25 (n = 9)

Values are means ± s.e.m.

In contrast to mammals, insects do not possess a closed, high blood pressure circulatory system. Therefore they lack the initial force present in the mammalian kidney which is responsible for the formation of a primary urine filtrate. They rely primarily on a diuretic hormone for the production of a rapid flow of fluid through the tubules. The response to diuretic hormone is dramatic in blood-sucking insects that feed infrequently[8]. Between feedings they carefully guard their water loss but on feeding they imbibe vast quantities of fluid relative to their size, and the surplus water is then rapidly excreted. The *in vitro* response to exogeneous diuretic hormone or to exogenous cyclic AMP is far less striking in plant-feeding insects, such as locusts, that feed regularly. Presumably their malpighian tubules are already somewhat stimulated and the increase in secretion as a consequence of their exposure to diuretic hormone or to cyclic AMP, is limited. Most of the insect's body fluid is circulated through the excretory system in a short span of time[2] and derangement of the orderly process of events could rapidly lead to fatal dehydration[4,5]. This is what happens when L-canavanine[4] or other non-protein amino acids[5] are injected into the haemolymph of the insect. On the other hand when insects are exposed to chronic doses of L-canavanine by feeding[3] death is due to the retention of water. It is clear that such apparent contradictions need resolving.

Malpighian tubules of locusts were isolated according to the method reported previously[9]. This involved the removal of the whole length of the gut with the tubules attached. The gut was bathed by a physiological saline medium in a wax-bottomed Petri dish and the whole preparation was maintained under liquid paraffin. The tubules were cut at their entrance into the gut and the cut end was extended around fine glass rods held by the wax. Drops of secreted fluid thus formed at the cut end and were subsequently measured.

Tubules were incubated in bathing media until basal secretory rates stabilised. Bathing media were subsequently changed to those containing L-canavanine, at a concentration of 10^{-4} M and secretory rates were monitored. The results are shown in Fig. 1 and compared to the performance of the tubules in normal *in*

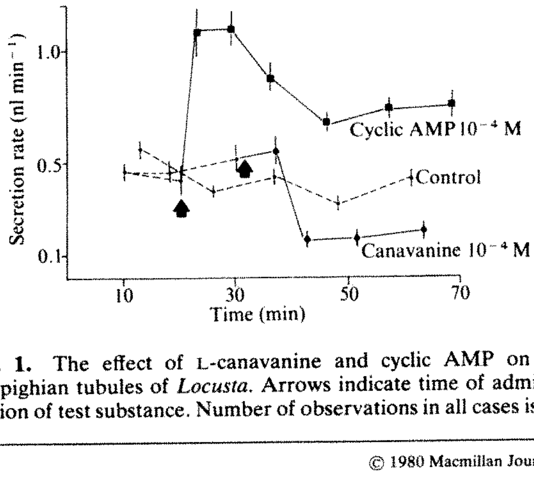

Fig. 1. The effect of L-canavanine and cyclic AMP on the malpighian tubules of *Locusta*. Arrows indicate time of administration of test substance. Number of observations in all cases is 10.

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vitro conditions and on addition of bathing media containing cyclic AMP. After 7 min of canavanine administration the rate of fluid secreted by the tubules drops by 72.7%. After this initial drop the fluid rate stabilises to a new basal level much lower than the normal original level. On the other hand, cyclic AMP stimulates fluid secretion almost immediately (3 min after administration) and the rate stabilises to a new basal level, higher than the normal original level. Inhibition is also evident when both cyclic AMP and canavanine are administered together in the bathing medium (Fig. 2). The cyclic AMP dose–response curve drops significantly when canavanine is present showing a substantial inhibition of tubular fluid secretion (32.6% inhibition at 10^{-3} M cyclic AMP).

In an attempt to determine whether canavanine inhibition is reversible, experiments were designed in which cyclic AMP was added to the bathing medium after treatment with canavanine. It was found that the tubules not only responded to the stimulatory effect of cyclic AMP but their response was enhanced to a remarkable extent (> 700% increase in rate) when compared to the normal response of untreated tubules (Table 1). Since cyclic AMP mimics the action of the naturally occurring diuretic hormone[1], we tested the response of the tubules to diuretic hormone in canavanine-treated and untreated tubules (Table 1).

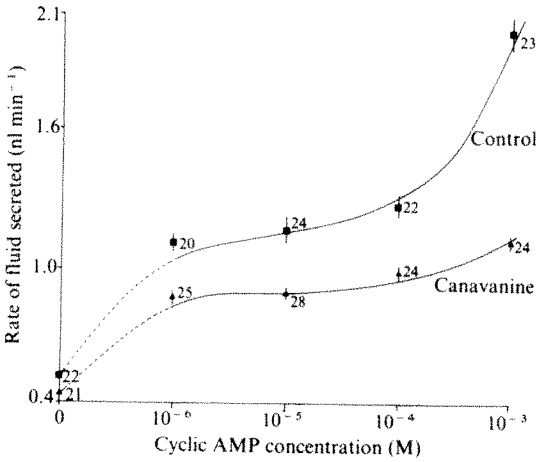

Fig. 2 The effect of L-canavanine on the normal dose-response curve of cyclic AMP. Subscript numbers indicate number of observations. Curves were fitted by eye.

It is clear that the diuretic hormone similarly stimulates canavanine-treated tubules to a greater extent (> 300% increase in rate) than untreated tubules. Thus canavanine, although in itself inhibitory, influences the tubules to react to a stimulatory compound much more effectively, possibly to the detriment of the insect.

We are now in a position to explain the apparent contradiction between the response of the whole organism to chronic exposure of canavanine (seen as a marked retention of water) and to a sequential exposure (seen as a loss of body fluid and dehydration). The inhibition of the isolated malpighian tubule by canavanine would lead to a marked retention of water. This is consistent with the retention of water which occurs *in vivo* in conditions of chronic exposure[3]. Since tubules themselves are capable of inactivating diuretic hormone[10], we assume that the inhibitor effect of canavanine occurs at a time when levels of endogenous diuretic hormone are much reduced. Canavanine apparently primes the tubules to respond to subsequent influx of diuretic hormone or to the generation of cyclic AMP. Indeed, the addition of diuretic hormone or cyclic AMP to tubules inhibited by canavanine elicits a dramatic increase in fluid transport, consistent with the dehydrated state attained *in vivo*[4,5]. This would only be true if exposure to canavanine is for a limited period which occurs when canavanine is administered by injection. Whether this priming is due to a general alteration in membrane transport properties is not yet clear.

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Use of cellulase antibodies to study leaf abscission

Roy Sexton

Department of Biology, Stirling University, Stirling, UK

Mary L. Durbin, Lowell N. Lewis & William W. Thomson

Department of Botany and Plant Sciences, University of California, Riverside, Riverside, California 92521

The primary leaves of the bean (*Phaseolus vulgaris* c.v. red kidney) are shed as the result of cell wall breakdown in the abscission zones found at the base of the lamina and at the stem–petiole junction. Horton and Osborne[1] suggested that cellulase (B 1 : 4 glucan 4-glucanohydrolase) was involved in this wall hydrolysis because its activity increased considerably during abscission. Although this result has been repeated on a wide range of plant material[2], critical scrutiny of the data has led several authors[3,4] to question the direct involvement of the enzyme in wall weakening. The following objections have been raised: (1) cellulase may be present at relatively high levels before abscission is induced[5], (2) although wall breakdown is confined to two or three layers of cells, cellulase is usually found over a wider area of the petiole, (3) the increase in cellulase which accompanies abscission may not take place until cell separation is essentially complete[3], (4) cellulase may increase without concomitant breakdown of the abscission layer[4]. One possible explanation for these anomalies is that these assays failed to distinguish between the various isozymes of cellulase, of which only one is postulated to be involved in abscission[6]. Recently, the specific isozyme thought to participate in bean leaf abscission (9.5 cellulase) has been purified and monospecific rabbit antibodies raised against it[7]. These antibodies do not cross-react with the other form(s) of cellulase and thus can be used both to quantify changes in 9.5 cellulase during abscission and to determine cytochemically the distribution of the enzyme. Using these antibodies, we have now made observations consistent with the participation of cellulase in the wall hydrolysis which leads to fracture.

Figure 1 shows a typical time course of abscission. At the time of induction the levels of total cellulase in the abscission zone can be easily detected. These increase dramatically after 18 h, when the force necessary to break the abscission zone declines. If the 9.5 isozyme is distinguished by radioimmune assay it is virtually undetectable in the uninduced tissue but starts to rise at 18 h just before a decrease in strength of the abscission zone. The correlation between the rise in 9.5 cellulase and the decrease in break strength is striking.

The antibody has also been used to localise the enzyme in formaldehyde-fixed cryostat sections (Fig. 2). This method reveals that 9.5 cellulase is confined to the two or three rows of

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cortical cells though which the fracture will pass and is also found in a wide band of vascular tissue. Unfortunately, nonspecific binding of antibody to the vascular tissue made interpretation of this observation difficult. To check these localisations, small fragments of tissue (100 μg) were dissected from the cortical and vascular areas of the fracture face and the cellulase content compared with adjacent areas outside the abscission zone. The stele contained very high levels of cellulase (2,012 units per g) in the zone compared with the vascular trace proximal to it (31 units per g). The abscission zone cortex also had elevated levels (167 units per g) compared with the general petiolar cortex (14 units per g). These viscometric assays are thus consistent with the immunocytochemical staining patterns showing 9.5 cellulase to be restricted to the fracture plane in the cortex. In the vascular tissue, cellulase is not limited to such a precise band of cells; this may account for the rather variable line of fracture in this tissue and objection (2) above.

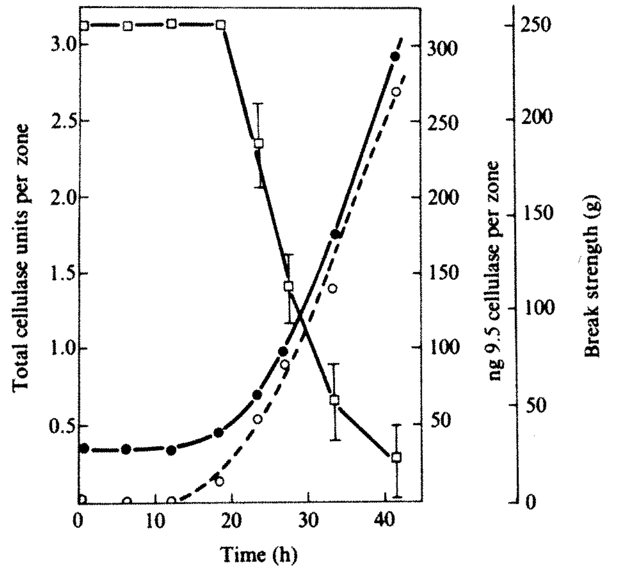

Fig. 1 A time course of abscission in the stem–petiole abscission zone of primary bean leaves. Eleven-day-old plants were induced to abscise by deblading 2.5 cm above the stem–petiole junction. The explants were placed in a chamber through which 50 p.p.m. ethylene was passed (1 l min^{-1}) in CO_2-free air. Samples of 70 zones were taken at the time points shown and the break-strength or force necessary to fracture the abscission zone determined[5] (□). Also, at each time point 70 zones were excised, extracted in salt-fortified buffer and total cellulase determined by a viscometric assay according to the methods of Lewis and Varner[5] (●). Radioimmune assay was carried out on the same samples (○). 9.5 Cellulase purified by the methods of Koehler *et al.*[7] was iodinated with ^{125}I using the chloramine T method essentially as described by Chard[8]. The iodinated cellulase was separated from its contaminants by chromatography on a cellulose column and elution by cellobiose[7]. The construction of a calibration curve and determination of unknown samples were carried out as described by Chard[8]. Results are all expressed on a per zone basis.

Thus, both the time course of 9.5 cellulase appearance and its distribution are completely consistent with an involvement in abscission. In an attempt to substantiate this role further, we injected serum containing 9.5 cellulase antibodies into the laminar abscission zone. It was hoped that the antibody would reach the exocellular cellulase producing the precipitin complex and so preventing its active involvement in wall hydrolysis. Two batches of 250 laminar abscission zones were injected with either 2 μl of normal rabbit serum or serum containing 9.5 cellulase antibodies (titre 4,900 units ml^{-1})[7]. This was achieved by passing the needle of a 'Hamilton' syringe along the centre of the petiolar pith cavity until the resistance of the pulvinus was met. At this point, the needle tip rests in the fracture plane in the vascular area where most cellulase is produced. Injections were made at 6-h intervals during the period when the 9.5 isozyme appeared, and then at 40 h the break strengths of the zones were determined. The zones injected with normal serum had an average break strength of 49 g (±26.1 g) whereas 90% of those treated with cellulase antiserum failed to break even if 200 g was applied. The 10% of this sample which did break in the abscission plane had mean break strengths of 167 g (±20.4 g). Thus, the injection of 9.5 cellulase antiserum had almost completely prevented wall breakdown. The levels of cellulase recovered from these cellulase antibody-treated zones was 24.4% of that in the normal serum controls. Presumably, this represents the enzyme which was inaccessible to the antibody, perhaps at intercellular sites in the tissue. As the antiserum has been proved to be specific to 9.5 cellulase[7], the fact that it stops abscission while the normal serum does not, confirms that cellulase has a central role in the abscission process.

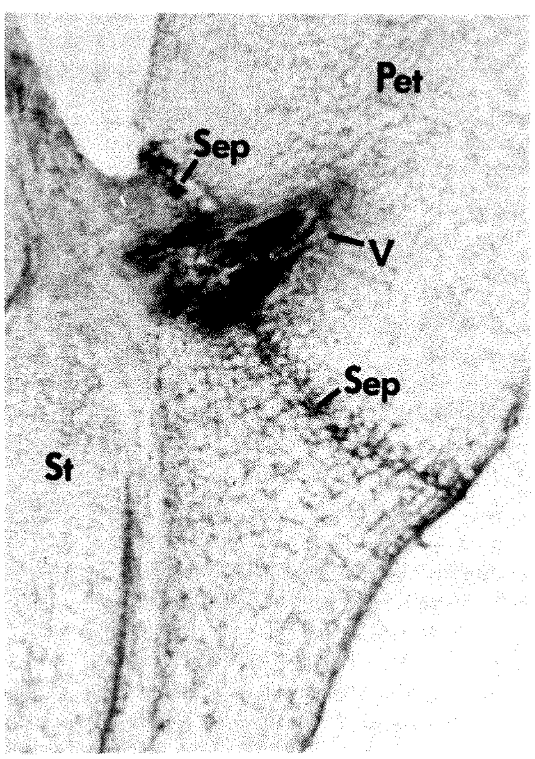

Fig. 2 A longitudinal cryostat section through the petiole base 36 h after the induction of abscission, incubated to show the distribution of cellulase. The enzyme was localised using the indirect method. Formaldehyde-fixed sections were placed first in rabbit anti-cellulase serum. After washing and denaturation of the native peroxidase[9], they were placed in diluted peroxidase-conjugated goat anti-rabbit immunoglobulin G. After further extensive washing, the bound peroxidase was localised by Graham and Karnovsky's method[10]. St, stem; Pet, petiole; Sep, separation layer; V, vascular trace. ×25.

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STUDENT BOOKS SUPPLEMENT

The lives of the spineless

H.C. Bennet-Clark

Lecture Notes on Invertebrate Zoology. Second edition. By M.S. Laverack and J. Dando. Pp.194. (Blackwell Scientific: Oxford, 1979.) Paperback £5.50. *A Life of Invertebrates.* By W.D. Russell-Hunter. Pp.650. (Collier-Macmillan: West Drayton, UK, 1979.) £12.75. *The Invertebrates.* By R. McNeill Alexander. Pp.562. (Cambridge University Press: Cambridge and New York, 1979.) Hardback £28; paperback £7.95.

THE teaching of the zoology of invertebrates, like their classification, has been the subject of the changing fashion. Major pedagogic problems are whether to lay emphasis on systematics or on comparative functional biology, whether to underpin the subject with comparative anatomy or with arguments about phylogeny. The subject is so vast and has such an extensive literature that any attempt to present, in a single textbook, a comprehensive account of the invertebrates is bound to be unbalanced and incomplete; one must therefore judge any text on the insight it shows and on the impact it will have on students of all ranges of interest and ability. Here, at once, we have three very different approaches, none exhaustive but all with contrasting merits.

The easiest to evaluate, because its aims are the least controversial, is Laverack and Dando's *Lecture Notes on Invertebrate Zoology*, now in a somewhat enlarged and welcome second edition. As the title implies, the work offers a series of brief descriptions of all invertebrate phyla, giving a diagnosis and brief description of the anatomy, aspects of the physiology, development and classification of each phylum.

For small groups such as the Echiura or Brachiopoda, this formula works well enough, though the former are described within the chapter as both Echiurids and Echiurida; and for the latter there is neither a description of the structure and composition of their shells, nor of their embryology nor, possibly wisely, a discussion of their affinities.

With the larger phyla, notably the Protozoa, Arthropoda and Mollusca, the treatment becomes less successful. That of the Protozoa, compressed into 11 pages, is so brief as to be confusing, while for the Mollusca a similar compression leads to over-brief treatment of the major classes Gastropoda and Lamellibranchia (surely Bivalvia?). With the Arthropoda the problems seem more deeply rooted. I find it hard to equate the larval forms and metamorphoses of Crustacea with those of Insecta and so I am unhappy to see these described together. Whatever one's views of the controversy, one cannot reasonably ignore the work of S.M. Manton on Arthropod phylogeny; but there is no discussion either of this or of the relations between the major Arthropod taxa.

On the whole this is a useful book. The pictures are borrowed but plentiful and well chosen, there are brief but handy lists of references — with notable omissions of some major works published in the last couple of years; the layout is good and the price is right.

Russel-Hunter's *Biology of Lower* and *of Higher Invertebrates* are old friends and deserved the success they achieved. Published about ten years ago, they now creak a little so this, their derivative, *A Life of Invertebrates*, is timely. The formula is similar; a series of chapters on aspects of the biology of different phyla — a couple on the Coelenterates, a couple on Crustacea, eight on Mollusca, one into which "Walking Worms" (Ugh! — guess the phylum), the Tardigrada, Linguatulida and Echiuroidea (!) are concreted. Between and within these are useful chapters, sadly under-played in the other texts: on metamerism in the Annelida; on the super phyla of the animal kingdom; on the phylogeny of the invertebrates; and so on.

At points the layout and balance are slightly suspect. Protozoa are considered in a single brief chapter and after the Coelenterates, which receive three chapters. The insects are skimped in a single long chapter and in rather less detail than the treatment accorded to gastropod or cephalopod molluscs. Against this, the text is mostly well laid out, written and illustrated; and, above all, the author manages to convey an admirable feel for particular phyla without congesting his text with turgid and repetitive detail.

Even though it is so clearly presented, I have reservations about this book, even as the elementary text it is designed to be. I find many of the diagrams — such as those of the tunicates (32-2) or of the tardigrade (18-2) — too sketchy, though other original diagrams are excellent. I cannot but contrast the clarity of Russell-Hunter's exposition on worm locomotion with his excessively thin description of protozoan locomotion, a topic which Alexander handles lucidly and in detail. Above all, I find it hard to recommend to my students any text from which access to primary sources is so difficult. True, there is an annotated bibliography at the end of the book but exclusively to textbooks. It would, for example, be hard to discover details of the functioning of crustacean endocrine systems or of the lamellibranch filter-feeding gill from any of the sources he cites, even though both topics are described by him in considerable detail.

In Alexander's *The Invertebrates*, there is no attempt to describe the diversity of major morphological characteristics of invertebrate phyla and, in his preface, Alexander writes: "This sort of information is available in existing textbooks and indeed dominates some of them so that they fail to reflect current trends in zoology". Those who know and respect his other books will expect to be enlightened and excited by his latest offering — and will not be disappointed.

Here are many insights into the operation of invertebrates; I have only space to select a few titbits. The description of how a flagellum works, though simplified, is up-to-date, rigorous and understandable. It extends to a comparison of forms in which the direction of swimming is the same as that of the flagellar beat with those in which they are opposite. There is also a nice comparison of the roles of geodesic fibres in different phyla — in nemerteans as constraints on both the maximum and minimum length obtainable, in nematodes as antagonists to the longitudinal muscles, and in the mantles of cephalopods as constraints on the length during jet propulsion; the first two, of course, are well known but it is nice to see the story extended and elaborated. The shells of molluscs are treated both as geometrical structures and as examples of skeletal materials. Where, in these descriptions, a series of calculations is required to substantiate the argument, the reader is led through a gentle series of O' level sums!

As Alexander is scrupulous in referring to his sources in text, in figure legends and in generous chapter bibliographies, it is easy to pursue topics further. But woe betide the student who, buying this book, expects to master the invertebrates. He will not find much on the systematics or relations between invertebrate groups and only an occasional and often highly specific account of anatomical features. It must therefore, to achieve its aims, be used in conjunction with more solid conventional text books.

I find the book enthralling and irritating. Enthralling because of the insights that are offered and the novelty of the approach. Irritating because it treats the different invertebrate phyla in an inconsistent rather patchy way, because the student may be seduced into believing that comparative systematics and anatomy may be ignored and, rather pettily, because many aspects of invertebrate biology that I had laboriously collected to gild the unleavened gingerbread of my teaching are here gathered together, augmented, under one cover. As a text, it may not endure but it will certainly be influential and is a 'must' in any reading list.

These are three very different treatments. All offer a welcome relief from the larger 'straight' invertebrate zoology textbooks. None, alas, is sufficiently comprehensive to stand on its own. Equally, each has its considerable merits and a place in any course in invertebrate zoology. □

H.C. Bennet-Clark is Lecturer in Invertebrate Zoology at the University of Oxford, UK.

Parasitological literature

R.W. Ashford

Medical Parasitology. By R. Leventhal and R.F. Cheadle. Pp.168. (F.A. Davis: Philadelphia, 1979.) $19.95. *Parasites and Western Man.* Edited by R.J. Donaldson. Pp.220. (University Park Press: Baltimore, 1979.) £12.95. *The Biology of Parasitism.* By P.J. Whitfield. Pp.277. (Edward Arnold: London, 1979.) Paperback £7.95.

PARASITOLOGY may be approached as an applied or academic subject, and the applied subject requires workers at all levels of sophistication. These three books span the field: an elementary practical text; a more specialised applied text; and a strictly academic volume in which apology is made for dealing in detail with those applied aspects which have contributed to understanding.

The self-instructional text by Leventhal and Cheadle is aimed at students of laboratory technology, medicine and biology. This volume differs from many works by including short-answer self-assessments at the beginning of the book and at the end of each chapter, and a final examination at the end of the book. The text describes the main parasites of man, using verbal rather than graphic life cycle diagrams, tables, diagrams of the diagnostic stage, and glossaries of terms. A section deals with laboratory techniques. Finally there is a collection of colour photographs illustrating many of the parasites. Although the book was evidently produced hurriedly, as indicated by the many typographical errors, much of the information and terminology is out-dated. The colour photographs do not bear comparison with those in a number of readily available books; they are sometimes strangely coloured, and the inaccuracy of some of the mangification scales will make them of little help in identifying real parasites.

A concise text with test questions could have been a useful aid to revision for examinations; it is unfortunate that this volume fails to meet the required standard.

Parasites and Western Man, produced by a team of eminent contributors, is aimed at medical and veterinary workers at all levels, particularly those with public health interests. In materially advanced Western society much of the public health problem concerns imported infection, but there are also surprisingly large numbers of parasites which hang on, and even thrive, in Western countries. In his introductory chapter, Nelson, provocative as ever, expounds the argument that parasites may have a positive role in human health, and that the search for a balanced relationship with them may be more valuable than blind destruction.

Each major group of parasites is covered in a chapter, and the ubiquitous head lice are given one of their own. Concluding chapters deal with the role of pets and domestic animals in human parasitic infections. The life cycles, modes of transmission, clinical and public health aspects, and statistics of occurrence of parasites in Western countries are outlined; and in the case of the more prevalent lice and mites, considerable epidemiological detail is given. More of this detail would have been useful for other parasites.

If this work has a wide enough circulation, it may help to control both the irrational horror which attends the discovery of a louse or pin-worm, and the equanimity with which the pollution of our residential areas by faeces from pet animals is commonly viewed. Despite a few slips, and the repetition inevitable in a multi-author work, this is a highly readable, thoroughly authoritative text which fills a gap in the literature.

Whitfield's *Biology of Parasitism* is a brave effort to summarise, for undergraduate and early postgraduate biologists, the outcome of technological and conceptual advances in the subject over the last ten years. The treatment of the whole subject of "Association Biology" in a "unified" language has allowed a broader view than is customary. Structure, nutrition, physiology, behaviour and ecology are reviewed in separate chapters.

The impressive breadth of subject covered has meant that depth has been limited; instead of indulging in broad generalisations, however, the author has achieved this by concentrating in depth on a few well-worked examples. In many cases a single association is viewed from different aspects in different chapters, and this gives continuity to a work which could easily have become disjointed. Recent information on surface interactions between parasites and hosts is covered in detail, and though the whole subject of parasite immunology is reduced to thirteen pages, the latest advances are summarised without gross oversimplification. The section on population studies is notable for the contribution which the author and his colleagues have made, but there remains an important communication gap between applied epidemiologists and the theoretical ecologists.

There is no attempt to be comprehensive or to supersede existing textbooks; this is a complementary work for advanced students. It will be particularly valuable as a basis for seminar discussions and as a source of wide-ranging up-to-date references. My only complaint is that the fashionable complexity of the language used, though meticulously accurate, will preclude the use of this book by many overseas students.

Three contributions to the sagging shelves of parasitological literature: our students, on appropriate courses, will be advised to avoid Leventhal and Cheadle, to at least read Donaldson, and to buy Whitfield. □

R.W. Ashford is Senior Lecturer in Parasitology at the Liverpool School of Tropical Medicine, Liverpool, UK.

Radioactivity in context

Malcolm C. Scott

Radioactivity: A Science in Its Historical and Social Context. By E.N. Jenkins. Second edition. Pp.197. (Taylor and Francis: Basingstoke, UK, 1979.) Paperback £4.75.

THE discovery of radioactivity and, subsequently, fission is one of the many fascinating stories associated with the development of science generally, and one which gives as valuable an insight as any into the nature of the scientific method. The general topic of radioactivity and its exploitation is also one for which it can be argued that there are moral issues involved, principally in the use of nuclear power. A book covering both these aspects should, therefore, provide stimulating reading.

It is therefore with regret that one records that the author of this book has not been able to meet the challenges presented in communicating either the excitement of discovery or the moral implications. Although the thirteen chapters cover a reasonably comprehensive spread of material, ranging from 'The Discovery of Radioactivity'' through "The Chemistry of the Fission Products" to "The Biological Effects of Radiation", the quality of the coverage is not very uniform. As the author was a research chemist until taking holy orders in 1962 it is perhaps to be expected that the chemistry sections are the most fluent. However, some of the other sections seem disjointed and even faintly dated, in the sense that developments since the book's first edition, in 1962, sometimes do not get the weight which workers active in the field would assign to them. Additionally, the advances made in the teaching and textbook presentation of scientific material in the 1960s and 70s are not reflected here: in fact, parts of the book are distinctly heavy going, and would hardly seem to appeal to the first-year university student, or equivalent, for whom the book is intended.

There are a number of minor errors and misleading statements which, though not central to understanding, leave one uneasy. For example, the different quenching mechanisms in a Geiger counter are confused (page 24) and the definition of neutron multiplication factor (page 107) cannot easily be related to and is inconsistent with the data given in the associated table. Perhaps the most disappointing aspect, however, is that moral and social questions do not get the depth of coverage which one would expect from reading the introduction. For example, what does the risks versus benefit balance sheet look like for the medical uses of radiation or for nuclear power? Are enough facts known for us to be able to draw up such a balance sheet? Can there be a qualitative difference between risks producing the same number of deaths in a given period of time and, related to this, what makes some risks socially acceptable whilst other, lower ones, are unacceptable? The reader looking for a discussion of these and related questions will not find them, yet these, surely, lie at the heart of any 'moral' evaluation.

Given the didactic philosophy behind the *Wykeham Science Series* and the excellence of some of the resulting books this one is somewhat disappointing. □

Malcolm C. Scott is a Senior Lecturer in the Department of Physics at the University of Birmingham, UK, in charge of the MSc course on the Physics and Technology of Nuclear Reactors.

Behaviour of practical systems

I.S. Grant

Transmission and Propagation of Electromagnetic Waves. By K.F. Sander and G.A.L. Reed. Pp.410. (Cambridge University Press: Cambridge and New York, 1978.) Hardback £24.50; paperback £5.95.

THIS book is intended to be used as a textbook in the later stages of undergraduate electrical engineering or applied physics courses. The emphasis is on the practical design and inherent limitations of the transmission lines, waveguides and antenna systems used in modern communications.

Familiarity with Maxwell's equations is assumed, and the book starts by deriving the plane wave solutions to Maxwell's equations. Boundary conditions are discussed, and reflection, refraction and absorption at interfaces between media are described with considerable mathematical elaboration. Guided waves in transmission lines and wave guides, and waves radiated from antenna, are all introduced as solutions to Maxwell's equations. I have some reservation about whether this approach is the most suitable for all students whose primary aim is to understand the behaviour of practical systems. But the treatment is not very formal, and the authors illustrate their argument with realistic worked examples. The first half of the book is completed with a standard treatment of transmission line theory, discussing reflections and impedance matching, and with a short section on the reflection of pulses.

The second half of the book is devoted to the propagation of communication signals in transmission line, waveguide and microwave systems. Attenuation and noise are considered for all the systems, and the characteristics of cables and waveguides capable of carrying signals over very long distances are described in considerable detail. The generation, reception and processing of signals is not discussed except where it is necessary as part of the explanation of how signals may be changed during propagation; modulation and multiplexing have to be understood, for example, in order to consider the signal degradation due to intermodulation when non-linearities occur in repeater amplifiers. The treatment of propagation is excellent — not difficult, but clearly explaining what is happening in terms of the basic principles given in the earlier part of the book. □

I.S. Grant is a Senior Lecturer in the Department of Physics at the University of Manchester, UK.

General geophysics

D.H. Tarling

The Physical Environment. By B.K. Ridley. Pp.236. (Wiley: New York and Chichester, UK, 1979.) £8.50.
Palaeomagnetism and Plate Tectonics. By M.W. McElhinny. Pp.357. (Cambridge University Press: Cambridge and New York, 1979.) £7.50.

RIDLEY'S is an interesting little book designed to provide an awareness of the total underlying physical structure of the environment for students of science and engineering. The scientists to whom the book is addressed include social scientists and it therefore uses a minimum of mathematics in its attempt to provide a unified basis for the comprehension of the relationships between astronomy, geology, meteorology and oceanography. The object is therefore laudible but possibly unattainable within such a small compass unless more rigorous mathematical treatment were introduced. This would then probably lose the innumerate section of his audience, exactly those people most likely to benefit from such a synthesis.

The contents are logically organised, starting in outer space and time, before considering the internal and then external features of the Earth. Halfway through there is a more general discussion of the forces of nature, including nuclear reactions, electromagnetic, chemical and gravitational forces, and then a return to the Earth's surface and the forces operating in and on the oceans. The book then begins a return to the outer limits of the Universe, discussing the Earth's atmosphere, solar and stellar processes and finally considers the origin of the Universe, the Solar System and life.

The tour is thus certainly comprehensive and there is little that can be considered either wrong or over-simplified to the extent of giving misleading impressions. It is thus difficult to pin down a feeling of uneasiness. Some of this almost certainly stems from the style, which is generally very readable but tends to launch into purple prose during either introductory or linking sections. Another reason for unease may be the balance given to different aspects. Four pages on Obler's paradox (and extra reference later on) seems excessively unnecessary when inertial waves are not discussed (only inertial oscillations) yet are virtually assumed within the consideration of wind patterns. Other unease arises from inadequate checking. For example, the age of the base of the Cambrian has different values on different pages. Such errors are trivial in terms of the context, but indicate a lack of care in proof-reading. Another unease is the occasional error — the magnetic dipole is "inclined" at 11° relative to the Earth's axis of rotation, but this state is to be known as declination.

A final cause for unease is the apparent reliance on the London Geological Museum for many of the geological-geophysical 'facts' and diagrams. While the Museum's exhibition is an excellent one, it is already out of date in some aspects, such as the presence of a basaltic layer beneath the continental granites — a feature that appears in almost all of the diagrams. It is similarly misleading, or indeed incorrect, to say that volcanic activity at subduction zones is due to the melting of light material thrust into the asthenosphere.

In general, the colour diagrams are excellent — mostly derived from the Geological Museum and NASA — but the black-and-white photographs are poorly reproduced, originally colour prints have poor contrast in grey tones, and it seems unnecessary to actually print the *Times* weather map with lettering from the reverse side still showing. The omission of the fold-out weather satellite photographs could have released money for improving other photographs. However, the diagrams are mostly clear and well produced, as is the book as a whole. There are problems at the end (with answers) for those who wish to test themselves; and there is further reading provided for each chapter, although I see no reason that the reading for chapter 5 is incorporated with that for chapter 4 and there is none for chapter 7.

Despite the qualms, it is possible to recommend the book for the general student scientist, although it is clearly designed for a British rather than a world-wide market. One hope is that this may be a short print; a revised edition would be likely to be more strongly recommended.

The second book, McElhinny's *Palaeomagnetism and Plate Tectonics*, is an entirely different proposition. This is aimed at a specific market, the (advanced) undergraduate geologist or geophysicist in particular. When it was originally issued in 1973, this was an excellent introduction to palaeomagnetism and a review of extant palaeomagnetic data. This "first paperback edition" is, in fact, a straight reprint of the hardback edition. Such a term implies to me that some editing or updating has been undertaken and as this is certainly not the case, the publisher's claim that it is "a review of the state of knowledge in the subject", which was justified in 1973, cannot conceivably be justified in 1979. The data base has expanded severalfold since McElhinny submitted the manuscript in, presumably, 1972. There have also been major advances in our understanding of magnetic processes within rocks, the behaviour of magnetic minerals, the instrumentation to measure either the magnetisation or other magnetic properties, and so forth. It is thus invidious to both the author and the reviewer to comment in detail on how certain aspects no longer correspond with current knowledge or awareness. CUP would have been better advised to issue a revised edition. As it is, this book can still be recommended as highly readable and well presented. The production is good and reasonably durable. It is with sorrow and disappointment that one can no longer give this paperback edition the recommendation which the original hardback deserved so well. □

D.H. Tarling is Senior Lecturer in Geophysics at the University of Newcastle-upon-Tyne, UK.

Solid state physics

W. Cochran

The Solid State. By H.M. Rosenberg. Second edition. Pp.274. (Clarendon Oxford University Press: Oxford, 1979.) Hardback £5.50; paperback £2.25.

THE first edition of this book appeared in 1975 and I feel sure that it was read and enjoyed by most lecturers in solid-state physics, and that they will have recommended it to their students. It is one of the Oxford Physics Series, which the Editor describes as core texts intended to cover material usually treated in the first year of honours courses in English universities, or in the second year in Scotland. The Editor introduces the book as one which succeeds in capturing the spirit of the stimulating lecture course that Oxford undergraduates have enjoyed for many years from Dr Rosenberg, and goes on to claim that it requires only a fairly basic background in mechanics, electricity and magnetism, and atomic physics, along with relatively intuitive ideas in quantum physics.

The only important difference between the second and first editions is the addition of a fourteenth chapter, on superconductivity. While I liked this chapter as well as any, I fear it will be beyond the scope of students — particularly of materials science and engineering — at the level for which the book is intended. One or two new problems have been added after the earlier chapters, and fourteen after the final one, to give a total of about 120. Numerical answers are now given where appropriate, although the author admits that he views the ensuing correspondence with some trepidation. A new section has been added at the end of chapter 11, to explain the phenomenon of saturation in NMR and ESR. Otherwise the second edition stays very close to the first. The opportunity has been taken to remove some minor errors: in particular Fig. 8.6 no longer conveys the impression that band overlap occurs at the zone boundary in one dimension. A remaining source of possible confusion is that while page 124 has "we shall use e for the modulus of the electronic charge . . . . .", in the section preceding a discussion of the Hall effect we read ". . . . where e is the electronic charge".

How does Rosenberg's book compare with the dozen or so others which a lecturer might recommend? The author's own list of 'standard texts', with his comments, is as follows:

Solid State Physics. By N.W. Ashcroft and N.D. Mermin. (Holt, Rinehart and Winston: New York, 1976.) A detailed, higher-level, modern treatment.

Solid State Physics. By J.S. Blakemore. (Saunders: Philadelphia, 1974.) A lively intelligent presentation.

Solid State Physics. By A.J. Dekker. (Macmillan: London, 1960.) A good introductory text.

Introduction to Solid State Physics. By C. Kittel. (Wiley: New York and Chichester, UK, 1976.) A standard text, brim-full with information and data.

Elementary Solid State Physics. By M.A. Omar. (Addison-Wesley: Reading, Massachusetts, 1975.) An introductory text which includes some unusual topics.

Physics of Solids. By C.A. Wert and R.M. Thomson. (McGraw-Hill: New York, 1970.) This is at a lower level than the other texts. It is very well written and illustrated.

Personally I would immediately delete Omar's book from any such list, as it includes a truly astonishing number of errors and misconceptions in its first few chapters, and I would add

Solid State Physics and Its Applications. By R.J. Elliott and A.F. Gibson. (Macmillan; London, 1974.)

Physics students in Edinburgh first meet solid-state physics in an introductory review course of 15 lectures given in the second year. The recommended text book is currently:

Real Solids and Radiation. By A.E. Hughes and D. Pooley. (Wykeham: London, 1975.)

A further thirty or so lectures are spread over the third and fourth years of the honours course. When I gave the third year course I recommended Rosenberg and Kittel, in that order, and in the fourth year I reversed the order. So to give the publishers a publishable quote — A book which we shall certainly continue to recommend to students in Edinburgh. □

W. Cochran is Professor of Natural Philosophy at the University of Edinburgh, UK.

Animal behaviour in laboratory settings

Trevor W. Robbins

The Experimental Analysis of Behaviour. By E. Fantino and C. Logan. Pp.559. (Freeman: Reading, 1979.) £8.40. *Stimulus Control of Behavior.* By H.K. Rodewald. Pp.162. (MTP Press: Lancaster; University Park Press: Baltimore, 1979.) £6.25.

FANTINO and Logan have undertaken the timely but formidable task of writing a student text which attempts to reconcile two often-opposed schools of thought in the study of behaviour: the formulation of general principles from laboratory experiments versus the ethological emphasis upon species-specific behavioural patterns, adaptation to ecological niche and evolution. Generally, they succeed admirably in this well produced and reasonably priced work which takes as its theme the dual ontogenetic and phylogenetic determinants of behaviour.

After a brief historical review of learning theory, basic concepts of operant and Pavlovian conditioning, as well as of "non-associative" learning such as habituation are described. Chapters on stimulus control, conditioned reinforcement, choice and aversive control then follow, but the rather staid headings belie the fascination of the topics subsumed. These include behavioural contrast, attention, concept formation, behavioural chains, self-control, punishment, self-punitive behaviour and "learned helplessness". There are also welcome allusions to the more successful applications of behaviour therapy such as the "token economy".

From these laboratory concoctions we are delivered quite smoothly across the 'Great Divide' to consider the niceties of courtship in snails and other behaviour in the wild. The ethological section is in many ways symmetric with the previous one, with chapters on species-specific behavioural patterns, "the natural history of learning" and two lengthy surveys of learning in invertebrates. The authors manage to cover taste aversion, specific hungers, imprinting, pigeon-homing, song-learning and the controversies surrounding the famous bee-dance. Considering such breadth, inevitably there are neglected areas. Thus, there is little on motivation, where one might perhaps have expected something on feedback control theory. Similarly, memory and claims of language-learning in animals are barely mentioned. I should have preferred rather less on learning in Protozoa to Platyhelminthes, where so much of the evidence is indecisive. On the other hand, important advances in the neurophysiology and analysis of learning in *Aplysia* are well represented.

The book is best suited for advanced students, as few punches are pulled when discussing theoretical issues. For example, the ramifications of Herrnstein's Matching Law are given an extensive quantitative treatment (though Rescorla-Wagner Theory is conveyed with not one equation!). The authors' boldness in incorporating the most recent material into their argument is also impressive, although the drive to synthesis sometimes obscures dissemination. For example, the critique of the use of 'anxiety' as an intervening variable to explain conditioned suppression seems mis-directed when couched physiologically. Neither was I convinced that satiety is "most likely" not a learning phenomenon (page 42), nor by the somewhat glib explanations of "vicious-circle" behaviour.

Minor blemishes are the typographical error which makes nonsense of Equation 7 page 241, and frustrating references to unpublished talks at meetings, sometimes by one of the authors (for example, page 283)!

However, the book is at its best in the concluding integrative section when the obvious merits of applying 'laboratory' techniques to ethological problems are made clear with examples taken from optimal foraging and displacement or adjunctive behaviour. Finally, the authors examine the "challenge to reinforcement theory". The challenge, with this book, seems well met.

Rodewald's book *Stimulus Control of Behavior* provides a striking contrast. Here is an unambitious tome which really does justify its perhaps puzzling description as a textbook for undergraduates or a "reference handbook for professionals who will find here a source of useful technology for the study of animal behaviour in laboratory settings". The subject-matter is probably of limited appeal, but is developed in easy steps in the classical pedagogical manner. The book is perhaps most suitable for advanced tuition in practical classes of operant conditioning involving experiments in psychophysics, attention, discrimination or memory. In his book, Rodewald cites some 159 references whereas the corresponding chapter of Fantino and Logan uses 133. It is perhaps of interest that these totals share only 14 references in common. Does compromise between schools of behaviour jeopardise concordance within them? □

Trevor W. Robbins is a Lecturer in Experimental Psychology at the University of Cambridge, UK.

Developmental psychology

James Russell

Human Development. By T.G.R. Bower. Pp.473. (Freeman: San Francisco and Reading, 1979.) $15; £8.40. *The Functions of Language and Cognition.* Edited by G.J. Whitehurst and B.J. Zimmerman. Pp.313. (Academic: New York and London, 1979.) $21; £13.65.

THE impression gained from reading these books in succession is that the aim of the Bower book is to review in order to teach and that of the Whitehurst and Zimmerman volume to review in order to present the case for modern functionalism. This is not to imply that Bower's book lacks a consistent theoretical standpoint; rather that the differentiation model which he espouses here, as in his other books, is such a broad one that there is much scope for eclecticism. Thinkers as different as Leibniz, Herbert Spencer, Piaget and, of course, Heinz Werner have presented differentiation models of development, and in Bower's case the framework allows him to support such diverse positions as neo-Piagetianism, social learning theory, and Laingian theory. There is much to agree with in his treatment of the standard developmental topics, but I think that his statement of the differentiation position as implying that concepts develop from the abstract to the concrete is needlessly paradoxical.

One of Bower's main neo-Piagetian themes is the notion that concepts and skills develop by being replicated on progressively higher levels. Given this, it is difficult to resist the facetious comment that Bower's own writings appear as points on a similar spiral: ideas explored in the early 1970s have now been presented a number of times each with slightly wider degrees of generality and very slightly wider data bases. It is a pleasure to encounter some of Bower's adventurous theorising at his current level (for example, the INRC group being first expressed in infant action) but less than a pleasure to find oneself noting how little of the referenced work has ever or will ever appear in journal form. Bower also evokes the same prissy reflexes by his too brisk treatment of certain topics: the evidence for the genetic basis of schizophrenia, personal construct theory are two such. But in general this book will make an excellent initial text for developmental courses so long as the course leader is at least as healthily sceptical as Bower himself.

Anyone who is at all familiar with the contents of the past three or four years of American developmental journals will feel at home in the Whitehurst and Zimmerman volume. The strength of the book lies in the fact that — as the editors point out — the writers were actively encouraged to make their contributions cohere. They succeed in the attempt, but given that most youngish American developmental psychologists have already been channelled towards a very definite stylistic and theoretical consensus I doubt if they had to try very hard. This is not meant negatively: the contribution of 'cognitive social learning theory' taken as a whole has been considerable even if the results of individual studies are seldom breathtaking.

Piagetian theory provided the impetus for much of the work reviewed here, but the Pragmatist tradition and experimental methodologies determined its orientation. Most of the contributors repudiate structuralism in cognitive (Piaget), linguistic (Chomsky), moral (Kohlberg) and memory (multistore models) development and most rely on the efficacy of social modelling, concept teaching and verbal rule learning in their arguments. There are many good things to be found: Ornstein provides an excellent comparative review of adult and child experimental work on memory and tries to show where cross fertilisation is and is not possible; Liebert directs a well aimed dart at the Kohlberg bubble; and Rosenthal forges some links between laboratory data and social and clinical problems. Overall this book will prove a useful text for advanced students and researchers who will find the rigorous referencing particularly useful. My only real misgiving is that the 'cognitive' in 'cognitive social learning theory' is being done less than justice here, as elsewhere. Why, after all, do modelling and training work? □

James Russell is Lecturer in Developmental Psychology at the University of Liverpool, UK.

Personality assessment

Graham F. Wagstaff

Personality. By R. Forgus and B. Shulman. Pp.434. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) £11. *Personal Enquiry and Application.* By M. Sherman. Pp.546. (Pergamon: Oxford and New York, 1979.) £7.95.

I AM sure that anyone involved with the study of personality will find these books valuable reading. Contrary to the approach adopted by a number of recent textbooks in the area, these volumes do not attempt to give an overview of a vast number of theoretical approaches, but attempt to view the subject matter from more specific perspectives. The authors recognise that the area of personality is confusingly diffuse and it is particularly difficult to select material, other than that concerned with descriptions of major theoretical approaches, that does not seem to give a superficial coverage of virtually all areas of psychology.

In the first two sections of his book Sherman provides a very useful and easily read critical presentation of methodology in the study of psychology, including the basic concepts of experimental design and analysis, correlation research, ethics of research, reliability and validity, and a review of interviewing, questionnaire, projective and behavioural techniques of assessment. In the final section he presents a critical discussion of psychoanalytic and cognitive learning approaches to personality, spending a considerable amount of space on the behaviourism/situationism issue. However, due to this latter emphasis detailed description of individual personality theories is very restricted, and those readers with a more phenomenological bias or who have interests in factor analytic trait theories, may feel that much of the extensive discussion of the principles of behaviourism is unnecessary and gives an unbalanced view of the issues in personality which is not specified in the title of the book.

Ironically, although Forgus and Shulman specify that their approach is a cognitive one, the reader may find that the content is more representative of the general area of personality than that covered by Sherman. The initial discussion seems to be more pertinent to the area of personality and includes guiding postulates for personality theory, and definitions and constructs used in personality theory. This is followed by critical overviews of a variety of personality theories including Freud, Erikson, Murray, Rogers and Lewin. It is not until chapters 5 and 6 that the cognitive orientation is clearly emphasised, and just as Sherman indulges in a description of behaviouristic principles, Forgus and Shulman engage in a discussion of the relationships of the perceptual system to need-related stimuli. In this way they attempt to construe personality within the context of motivation and the way events are perceived. Consequently they reinstate the concept of motivation as a primary concept in the study of personality. This analysis is then applied to personality development, interpersonal behaviour and psychopathology. Although Forgus and

Shulman's analysis is extremely well formulated, the breadth of the subject matter may still leave the reader very unsure as to the boundaries of the area, and for further reading he may decide that he had better read up all areas of psychology.

In spite of the wide content area, the factor analytic approaches to personality are again conspicuously absent. I find the absence of discussion of the theories of Cattell and Eysenck in both books rather puzzling in view of the pertinence of these approaches to issues involving the nomothetic trait and type concepts of personality, the extensive experimental work that has been conducted in relation to them and the ubiquitous presence of studies using the 16PF and the EPI. I would have thought Eysenck's behaviouristic analysis would have been particularly appropriate to Sherman's discussion of the behaviourism/situationism issue.

Both books assume little previous psychological knowledge of the reader and are easily comprehended. However, I feel that they will be of most value as supplementary, rather than main texts in personality. In this role both are sufficiently individualistic to assume equal prominence. □

Graham F. Wagstaff is Lecturer in Personality and Social Psychology at the University of Liverpool, UK.

Surveying microbiology

D.G. Smith

Biology of Microorganisms. By T.D. Brock. Third edition. Pp.802. (Prentice-Hall International: Englewood Cliffs, New Jersey and Hemel Hempstead, UK, 1979.) Hardback £17.15. *The Life of Yeasts.* By H.J. Phaff, M.W. Miller and E.M. Mrak. Second edition. Pp.341. (Harvard University Press: Cambridge, Massachusetts and London, 1979.) £10.50.

In these days of multi-authorship of textbooks, it is becoming increasingly rare for a major textbook to have a single author. T.D. Brock's production of three editions of *Biology of Microorganisms* within a decade must therefore be acknowledged as a remarkable achievement and it confers the advantage of a uniformity of style throughout the work.

The third edition follows the general format of earlier editions: the pages are large and attractively laid out although often wasteful of space. The subject coverage remains comprehensive and has been extensively updated to cover recent developments in areas such as recombinant DNA and bioenergetics.

A welcome addition to this edition is the incorporation of what the author calls "vignettes" on historical aspects of microbiology. These occur throughout the text labelled "a bit of history". Although these sections can easily be skipped, an appreciation by students of the historical development of a piece of knowledge is certainly to be encouraged.

Another feature of the new edition is the inclusion of five appendices (42 pages) containing detailed information on energy calculations, mathematics of growth, biochemical pathways, bacterial classification and microscopy.

This book is one of only two or three available titles which can be recommended as the main textbook purchase by undergraduate students of microbiology.

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This book is suitable for the non-specialist at school or college but there is also an appendix containing reference material on classification and characteristics of yeast genera which will be useful to the specialist. □

D.G. Smith is Senior Lecturer in Microbiology in the Department of Botany and Microbiology, University College London, UK.

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Electromagnetic quintet

D.S. Betts

Electromagnetics. By S. Seely and A.D. Poularikas. Pp.790. (Marcel Dekker: New York and Basel, 1979.) £14.40. *Electromagnetic Fields.* By A.M. Portis. Pp.775. (Wiley: Chichester, UK, 1979.) £16.30. *Electromagnetic Fields.* By R.K. Wangsness. Pp.576. (Wiley: Chichester, UK, and New York, 1979.) £10.75; $20.95.*Electromagnetism: Principles and Applications.* By P. Lorrain and D.R. Corson. Pp.507. (Freeman: Reading, 1979.) Paperback £7.30. *Electricity.* By C.A. Coulson and T.J.M. Boyd. Second Edition. Pp.392. (Longman: Harlow, UK, 1979.) £6.95.

THE first of this quintet is a big book, physically big and big in ambitions, intended for science and engineering students approaching graduation. Seely and Poularikas have set themselves the worthwhile task of developing electromagnetic theory and then applying it within an unusually broad context. This had led to the inclusion of "certain details of acoustics, thermal fields, plasmas, elasticity, physical and geometrical optics". So far so good. The trouble is that these details are rather too often given undue prominence. For example, Chapter 13, "Resonance, Resonators and Coherence", contains four sides of largely mathematical text devoted to the mechanical vibrations of strings and membranes. And in Chapter 8 on wave phenomena the reader finds himself wading through eight sides devoted to longitudinal waves, including a more complete proof of the velocity of sound in gases than is to be found in many basic texts on waves. I feel that in this respect the authors should have exerted a little more self-discipline.

On the other hand there are some very good things. Chapter 7, "Interactions of Charged Particles with Electric and Magnetic Fields", is extremely good, leading naturally into plasmas and the design of fusion reactors. And Chapter 12, "Elements of Diffraction Theory", gives thorough coverage to a tricky topic; and I also like much of the treatment of waves in unbounded media (not mediums, please!) and guided waves.

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The development of the basic equations and ideas of electromagnetism is fairly heavily loaded with references to non-electromagnetic phenomena and this seems to work reasonably well. Three minor quibbles: (1) On pages 236 and 367, it is surely wrong to regard $\underline{\nabla}\times(\underline{\nabla}\times\underline{X}) = \underline{\nabla}(\underline{\nabla}\cdot\underline{X}) - \nabla^2\underline{X}$ as a vector identity (except in Cartesian coordinates) — it is a definition of $\nabla^2\underline{X}$. (2) The satisfying proof given that current loops experience dipole-like torques in applied fields does not depend on the loop being plane, so it was a pity to preface the proof by the statement that $\underline{m} = I\underline{S}$. (3) It is doing Maxwell's equations an unjustice to describe (page 363) the charge continuity equation as auxiliary to them — it is built in.

In short, this is a valuable but somehow unwieldy book. It is not helped by its appearance with its typewriter print and spidery diagrams.

In Portis' *Electromagnetic Fields,* again there is à stated aim of putting a considerable amount of applied physical material in with the basic electromagnetic theory. Portis argues that this is more necessary than it was ten years ago because of the decline in the fraction of physics majors (in America) going on to graduate school. It is an argument whose application to the British scene is less than clear. Anyway, the result is a most attractive textbook but one which is fairly expensive and rather too wide-ranging to make large sales in the UK. "The selection of topics has been made within the context of a serious attempt to develop the classical theory of electromagnetic fields from a contemporary point of view." Some of these topics are flagged as being more advanced or specialised (for example, helicons, magnetic dichroism, Aharonov-Bohm effect) and the pages beyond 600 are given over to (valuable) appendices. So the 'core' of the book is in fact of quite manageable proportions, with each of the 15 chapters having a summary and generous number of both exercises and problems. It is well produced with only one noticeable oddity (the text goes down to within a quarter of an inch of the bottom of the page). In reading it, I appreciated the system of numbering topics within each chapter and it was easy to find my way around in. The parts which deal with straightforward electromagnetic theory are organised in a fairly conventional way, with a heavy dependence on vector manipulation in some sections.

Wangsness offers *Electromagnetic Fields* "for use in the usual year-long course in electromagnetism at an intermediate level given for advanced undergraduates". This is a straightforward aim and the text is a good conventional exposition of the subject, with few frills, up-to-date applications or fancy notions. Putting down Portis and picking up Wangsness one is aware of an enormous difference of attitude and approach. Wangsness gives a comforting feeling of old-fashioned virtue and I hope there is still a market for this. The chapters progress through Coulomb's law, the electric field, Gauss' law (so good to see the apostrophe correctly used), the scalar potential, and so on, in an orderly route which so many of us recognise from lecture courses and other texts. This is not intended as an unkind criticism because I rather like this book with its straight-laced academic air. There is, however, a dilemma: if you write a conventional book on a long-understood topic, in what ways is it better? I think the exercises are a particularly strong point in this case, with 130 actually worked out in the text and another 555 for the student. And the pointing out of common sources of error is helpful. On page 52, for example, the recipe for keeping source points and field points distinguished is good. In short, this is a conventional book, containing very little which could not have been written 50 years ago, but it is done thoroughly and with attention to detail.

It is important not to confuse this book by Lorrain and Corson with another on the same topic by the same authors but entitled *Electromagnetic Fields and Waves* (Freeman, San Francisco, 1970) abbreviated EFW below. The expressed aim is that the present book (*Electromagnetism,* abbreviated EM below) be used at freshman or sophomore (first- or second-year) level "either as an introduction to the subject for physicists and engineers, or as a last course in electromagnetism for students in other disciplines". I presume that those going further would find themselves at home in the other book, EFW, of which I have a good opinion. The present book, EM, I find very satisfactory, with some sense of excitement about it. The examples chosen are often of an applied and modern nature which gives a slight advantage over Wangsness, and there are three chapters on alternating currents which Wangsness leaves out altogether. On the other hand there is only one 22-page chapter on electromagnetic waves, which both Wangsness and Portis are strong on. The general level of production is excellent and the diagrams and problems are admirable, and I can see huge advantages in having a more advanced relation (EFW) to help both authors and readers over sticky patches. In such places, there are footnotes giving references to appropriate points in EFW. The price is, by today's standards, quite modest. It is an attractive book, obviously designed for a large market which I think it deserves to win.

The original version of *Electricity,* by Coulson alone, was published in 1948, and it was used successfully by undergraduates at Oxford and elsewhere for a considerable period. My view of it, as a student, was that it was helpful but rather highly condensed and therefore tough-going. Looking at the old edition now, it is clear to me that the difficulty was mainly that I was a physicist and that it was conceived and published as a mathematical text. Thus, there were very few references to experiments or to applications. The new edition has been prepared by T.J.M. Boyd, an applied mathematician, and I find it a very good job, though I would retain the reservation I mention above. There are noticeably more references to physical phenomena and particularly to plasma behaviour (all the five texts reviewed here give some discussion to plasmas). The units have been converted to SI and some minor improvements of style and coverage have been incorporated. But the main additions are the substantial four chapters at the end, on electromagnetic waves (Chapter 9), waves in bounded media (Chapter 10), the generation of electromagnetic radiation (Chapter 11) and relativistic electrodynamics (Chapter 12). These are good solid chapters which Professor Boyd has managed to write in such a way that the reader does not experience a style transition on beginning them. The work thus retains a sense of unity. Another improvement is in the use of computer methods for a variety of plotting tasks in which the results and the programs are given. The book is well produced and very reasonably priced. ☐

D.S. Betts has been a Lecturer in Experimental Physics at the University of Sussex since 1965.

General relativity

D.J. Raine

General Relativity and Cosmology. By J.V. Narlikar. Pp.279. (Macmillan: London, 1979.) £9.95. *General Relativity.* By J. Foster and J.D. Nightingale. Pp.192. (Longman: London and New York, 1979.) Paperback £5.45.

In June 1922, at the height of the deluge liberated by Eddington's confirmation of the bending of light by the Sun, *Nature* reviewed no less than seven books on relativity. In the whole of 1931 it reviewed one. Recent years have witnessed a revival of interest in relativity and a concomitant flux of literature at all levels, expecially as part of the centenary celebrations.

Einstein's theory is now half as old as Maxwell's electrodynamics. The fundamental theory has not changed, but much has happened to make the early expositions inadequate. First of all there has been a general diffusion of mathematical ideas, so that extended apologia for departures from Euclidean geometry, or for the mythical difficulties of the theory, are no longer necessary. Secondly, and paradoxically perhaps, with the hindsight of relativity we can now see Newtonian theory in a new way and general relativity can emerge as a necessary consequence of the special theory. Indeed, the teaching of general relativity would be so much easier if only Newtonian gravity were taught, as it so easily could be, in terms of the equivalence principle. But perhaps the most important change, already clear in the established modern texts, is the relentless strengthening of the empirical foundations of the theory which, despite the proclamations, was not finally confirmed by Eddington's expedition. This has culminated in the centenary year in the observations of the binary pulsar, which effectively confirm the existence of gravitational radiation, and play for general relativity a role similar to that of the Hertz experiment for electromagnetism. Thus general relativity has become as much a part of physics as Maxwellian electrodynamics — except that it is not useful for anything — and might be expected to give rise to a corresponding diversity of textbook treatments.

Professor Narlikar chooses to emphasise the physics of general relativity using only that mathematical dress that is decently necessary for proper calculation — no hint of a dual vector space, no definition of a differentiable manifold because, in the traditional expositions, these are not essential for local computations. Foster and Nightingale opt for a more mathematical approach which brings clarity to the structure of the theory but means that, within their limited space, they cannot go much beyond the classical results. Both assume an acquaintance with special relativity, although Foster and Nightingale give a review of this in an appendix.

Narlikar's book starts with a misprint in a sentence which is wrong anyway: how boring it must have been to write this opening! I was prepared to gird up my prejudices. But, in fact, most of the book is a masterpiece of pedagogic lucidity. Symmetries of spacetimes are dealt with properly in terms of Killing vectors, the theory of orbits in the Schwarzschild solution is developed in terms of effective potentials, Price's theorem in the collapse to a black hole and the four laws of black-hole dynamics all receive a mention. There is even space for derivations of the classical cosmological tests. It is a pity that the Robinson-Carter theorem on the uniqueness of black-hole solutions is missing, and that the results from the binary pulsar presumably came too late for inclusion. And the discussion of the black-hole candidate Cygnus X-1 is unfortunately, to put it politely, slightly confused.

Where I really feel obliged to take issue, however, is with the references to current areas of research activity. It is true that what is intended is only a glimpse and not a survey, and nowhere are they explicitly claimed to be representative. It is true also that most authors avoid the potential embarrassment of Time's sickle compass by leaving out where in doubt. The lecture format helps here (and here only) in lending an impression of informality. Nevertheless, I feel that Professor Narlikar comes dangerously close to confusing for the reader those matters of personal interest and opinion and the consensus view. The general consensus, right or wrong, is, for example, that quasars are cosmological, that the Hoyle–Narlikar theory is neither in accordance with Mach's principle nor even a putative solution to the problem of the existence of spacetime singularities, that the Steady State Theory is under something rather more substantially earthly than 'a cloud', that the Kaluza–Klein five-dimensional theory is awaiting only the funeral arrangements and that it and indeed f-gravity are less likely to produce a unified field theory than the unmentioned supergravity theories, and that interesting progress is being made in quantum gravity by evaluation of the path integral. The uninformed reader may well be left with a false impression of the main thrust of current thinking, which is a pity in such an otherwise admirable exposition.

The main danger in a mathematical approach to the elements of the theory is that general relativity emerges from the differential geometry as one amongst many possible theories, the geometrisation of gravity appearing as pure artistic creation or conventionalism. The idea that the equivalence principle drives one to this vision gets lost somewhere in the multilinear mappings. On the other hand, Foster and Nightingale do find space for explicit calculations as well as general results, so that a feeling for the inner working of the theory is communicated. The numerous exercises for the reader help in this, being used here to illustrate the theory rather than to develop it further as in Narlikar's book.

The special aspect of both of these books that distinguishes them from the established modern works such as those of Weinberg and of Misner, Thorne and Wheeler is brevity (a distinction which in relation to the latter is fairly universal). Of course, brevity of exposition does not guarantee greater rapidity of digestion. In fact, Narlikar achieves a masterly conciseness, and Foster and Nightingale

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concentrate on a proper mathematical development of the classical results.

But for a real example of how to write concisely we have to turn to the appendix of Foster and Nightingale. There we are told that if Newtonian dynamics and not special relativity were true, the two mile Stanford linear accelerator would need to be only one inch long. In a nutshell here is the essence of the status of special relativity; no technological details, no experimental arrangements, for these can be found elsewhere, but a correct overall view in a single picture. Would that it were all so simple. □

D.J. Raine is Lecturer in Theoretical Astrophysics at the University of Leicester, UK.

Basic chemistry

C.A. McAuliffe

Introduction to Modern Chemistry. By E. Meyer. Pp.527. (Prentice-Hall: Hemel Hempstead, UK, 1979.) £11.65. *Chemistry: A Conceptual Approach.* By C.E. Mortimer. Pp.815. (Van Nostrand Reinhold: Wokingham, UK, 1979.) £7.50. *Basic Chemistry.* By G.H. Miller and F.B. Augustine. Pp.420. (Harper and Row: London and New York, 1979.) £6.45. *Chemistry for the Consumer.* By W.R. Stine. Pp.496. (Allyn and Bacon: Boston, London, Sydney and Toronto, 1979.) £13.55. *Fundamentals of Chemistry.* By F.H. Redmore. Pp.711. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) £12.30.

I RECENTLY heard it said that one reason graduates from American and Japanese universities are more adaptable when entering industry than are UK graduates is that they benefit from a relatively non-specialised, more inter-disciplinary course than that which UK students enjoy. I think that there may be something in that notion. Certainly, I am, after seventeen years of fairly intimate contact, still amazed at the job which US universities do for the disparate student-entry they receive. The books reviewed here demonstrate quite well, in what they set out to do, just what a varied body they are aimed at. Indeed, it is a mistake for persons not familiar with US colleges not to realise that there is immense variation in college entries both in terms of preparation and ability of freshmen students. This is directly related to standards in US high schools, where teachers are frequently qualified in education but ill-qualified in the disciplines they teach, and also to the fact that since almost all high school students graduate and are thus, in many States, qualified for entry to State universities, the intellectual abilities span the whole spectrum. In turn, the requirement by many universities for students to pass a basic science course sets a daunting task for would-be authors of basic chemistry texts, but one that can be professionally and financially very rewarding for those who are successful.

Three of the books discussed here are directed towards students who either have never before had a chemistry course or have had very small exposure to chemistry. The text *Introduction to Modern Chemistry* by E. Meyer has for its first sentence in the Preface: "Teaching a course in basic, modern chemistry is often a unique educational experience for the instructor and students alike." It might well be a rare educational experience for a student to teach a course in basic chemistry, but if one should attempt it this is not the text to refer to other students. I think it is sloppy in Chapter 1, where the word gas is carefully defined a number of times, to also to refer to a gas guage (meaning gasoline guage). The introductory chapters seem to be written at an extremely elementary level. Despite my introductory paragraph, I feel it is insulting to any college student to include a half-page illustration of a mediaeval king with a pipe running from his nose to his finger tip to say that early standards of length had some anatomical origin.

There are two and a half pages of photographs of balances (pp. 34–36), and in the worked Example 2-4, one-third of a page is needed to convert 98 mm to 9.8 cm. On page 63 of this college chemistry text it is stated "Each element is associated with a unique symbol . . . . In place of carbon, we write C; for chlorine, Cl; for sodium, Na; and so on." Yet, only a little later (pp. 110–125), the Bohr model of the atom and quantum mechanical model are discussed.

It appears to me that authors such as Meyer should ask themselves just who they really are writing for. It is not conceivable to me that one can aim introductory chapters at students who cannot be numerate and are slow to learn, and expect these people to go so quickly from converting °C into °F to a discussion of the emission spectrum of the hydrogen atom. These same criticisms apply to *Basic Chemistry* by G.H. Miller and F.B. Augustine. On page 11, worked Example 2.1 takes two-thirds of the page to calculate how many centimetres there are in 25.00 m. Worked Example 3.3 answers the question "What is the mass of a chromium atom" with the unhelpful: "It is 52/12 the mass of a carbon atom." On page 105 the section on oxidation numbers is inconsistent and downright misleading. As in many such texts there appears to be poor judgement as regards what it is that is important for a chemistry book to contain; for example only two pages are devoted to the chemical properties of hydrocarbons.

The third of these volumes, *Fundamentals of Chemistry* by F.H. Redmore, is to be recommended. It does contain the trivial chapter (centimetres into inches), but after that it does assume the reader is of at least average intelligence and, in contrast to the Meyer and Miller/Augustine books, it is tightly written, and, like a number of good freshman chemistry texts, is able to simplify quite difficult theoretical concepts. Thus, simple molecular orbital and crystal and ligand field theory are very well presented. The structure of the atom is introduced early (Chapter 4) and is nicely done. I particularly liked Chapter 21: Periodic Variations. Redmore deals with the Born–Haber cycle — the first two books do not.

The fourth edition of *Chemistry. A Conceptual Approach* by C.E. Mortimer is, we are assured in the Preface, "the most student-oriented edition" yet. (Whatever next? Orienting textbooks towards students!) But it is a good book and deserves to have lasted so long. Chapter 2 on atomic structure is excellent, and I also particularly liked the chapters on kinetics and thermodynamics. The standard of text and diagrams is high almost throughout (but I did find that molecular orbital schemes using circles for s orbitals and squares for p orbitals was intrusive and detracted from the otherwise well produced figures). There are some more serious objections — wave mechanics is dealt with beginning on p. 41, whilst the concept of a mole is not introduced until p. 152; the student who can understand simple wave mechanics surely doesn't need to have explained what the term molecular weight means (also on p. 152). I also feel that standard and non-standard states need clearer definition.

Chemistry for the Consumer by W.R. Stine is aimed at the non-scientist and intends to provide enough chemical knowledge and principles for an understanding of such concepts as nuclear and radiochemistry, agricultural chemistry, food chemistry, medicinal chemistry and pollution problems. It just does not quite make it. I find it hard to imagine just to whom the book will appeal. If Stine would write such a book for the scientist he might achieve a more worthwhile object. I think this book has a most severe drawback; figures containing rather complicated chemical formulae coupled, albeit, with simplistic text, does not make for an understanding of chemistry by the non-scientist. Furthermore, although Stine says in the Preface that he hopes the reader will "gain a better understanding . . . of such things as energy, food, . . ." only passing reference to nuclear energy is made and the most important energy-capturing chemical system, photosynthesis, is briefly mentioned in the production of carbohydrate — but the non-scientist will probably not realise that photosynthesis converts solar energy into chemical energy. The word energy does not appear in the index. ☐

C.A. McAuliffe is Reader in Chemistry at the University of Manchester Institute of Science and Technology and Adjunct Professor of Chemistry at Auburn University, Alabama.

Unifying concept in organic chemistry

R. C. Poller

Organic Stereochemistry. By Henri Kagan. Pp.166. (Edward Arnold: London, 1979.) Paperback £4.95. Translated from *La Stereochimie Organique.* (Presses Universitaires de France, 1975).

ENGLISH LANGUAGE texts of organic chemistry are markedly derivative, so one might expect Professor Kagan's book to contain some fresh insight into a subject where the French contribution has been central. The reader will not be disappointed. Stereochemistry is seen to be a unifying concept in organic chemistry and it is satisfying to see a diverse range of topics from conjugation, through kinetic versus thermodynamic control, to pericyclic reactions discussed from this single standpoint. The treatment is from first principles and as rigorous as could be expected in a work of this size, though only a brief introductory account of the more marginal topics is possible.

All who teach stereochemistry will have been grateful for the moves toward a more logical and consistent terminology which have been evident in recent years. Substitution of the term "chirality" for the anthropomorphic "handedness" is allowing the too vague "dissymmetry" and the often misapplied "asymmetry" to fall into disuse. The clear division of stereoisomerism into enantiomerism and diastereoisomerism has liberated us from having to tell our students that the optically inactive meso tartaric acid is an optical isomer of the (+)-form. We no longer have certain cyclic compounds uneasily carrying the banners of both optical and geometrical isomerism and the universally applicable RS/EZ system of configurational notation is especially welcome.

We cannot expect that a text originating in a foreign language should simultaneously present novel viewpoints and a sensitivity to the English nomenclature. Kagan and his translators have chosen to use a mix of the old and new terminology. The deliberately literal rendering of the original has, nevertheless, led to some unfortunate semantic choices, for example, where the usual meanings of the terms "racemic compound" and "racemate" have become exchanged. Finally, although this is an inexpensive book, one has the right to expect a higher standard of proof-reading and, more importantly, better diagrams. The latter are small, not always clear, and occasionally confusing.

Despite these caveats this is a valuable book which is commended to all those who have an interest in organic stereochemistry. ☐

R. C. Poller is Reader in Chemistry at Queen Elizabeth College, London, UK.

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Inorganic chemistry

H. A. O. Hill

A Theoretical Approach to Inorganic Chemistry. By A. F. Williams. Pp. 316. (Springer: Berlin, Heidelberg and New York, 1979.) DM98; $53.90.

To my surprise, I greatly enjoyed reading *A Theoretical Approach to Inorganic Chemistry.* Usually when I hear 'theoretical' and 'inorganic' used in conjunction I reach for my revolver. However this admirable book is so full of good sense that all previous prejudices were overcome.

The first chapter contains the essence of quantum mechanics and atomic theory. The only constitutent lacking is the relationship between complex eigenfunctions and the angular momentum operator. The concepts culled from quantum theory are then used to describe polyelectronic atoms and ions, laying to rest in passing such a shibboleth as 'the stability of the half-filled shell'.

The key features of molecular orbital theory are succinctly described in Chapter 2 of which a salutory section discusses hybridisation. The application of molecular orbital theory to the interpretation of the structures of molecules is the subject of Chapter 3 and how best to begin than with a discussion of Walsh diagrams. The section on transition metal compounds is interesting though I was sorry to find the ligand field parameters, Δ and 10Dq, treated as if they are necessarily synonymous; and even more to find the Jahn-Teller effect introduced on page 82 without a proper statement of the theorem. However, the author makes some interesting comments on organometallic compounds, not least the description of the "eighteen-electron rule as an example of electron counting rules that work for no especial reason".

The discussion of electron-deficient compounds (or rather 'compounds for which the theory previously provided was deficient') and cluster compounds is very valuable. It was good to find the same ideas applied to solids, often neglected in popular modern texts. In the chapter on electronic and magnetic properties, the ideas of C. K. Jorgensen find a suitable home; they have long deserved wider exposure. The Jahn-Teller theorem is now discussed more rigorously and as a bonus (?) a brief mention is made of the second-order effect. It is a pity that the author, having kept firmly to SI units in the rest of the book, reverts to c.g.s. units when discussing magnetic properties.

An important chapter contains an account of alternative methods and concepts. Whether the X-method warrants its position at the beginning of the chapter is debatable. The valence bond model and ionic model are well treated and the section on Valence Shell Electron Pair Repulsion should be essential reading for all undergraduates. It is hard to think of an idea that has gained such wide yet uncritical acceptance. The section on "thermodynamics and inorganic chemistry" seems a little out of place in this company especially as it is followed by "Useful Concepts in Inorganic Chemistry". Some might take issue with that title since it includes a description of 'Hardness and Softness' which even warrants a subsection on 'theoretical aspects'. Still, as the author states, "as with other concepts in inorganic chemistry, we should know when they lead us astray". I found the account of "Mechanism and Reactivity" and "Descriptive Chemistry" valuable but that on "Physical and Spectroscopic Methods" less so though even here, the words, though few, are wise. Each chapter has an excellent bibliography.

I have no hesitation in recommending this book to both those who teach and those who study inorganic chemistry. This is especially so for those about to take examinations though by this time it may be too late for the remedial properties of the book to take effect. We should be grateful to the author for sharing his insight with us. □

H. A. O. Hill is Lecturer in Inorganic Chemistry at the University of Oxford, UK.

Physical principles of photochemistry

G.S. Beddard

Principles of Photochemistry. By J.A. Barltrop and J.D. Coyle. Pp.213. (Wiley: Chichester, UK and New York, 1979.) £4.95.

THERE are now available several photochemistry textbooks; their emphasis is on photochemical reactions but they deal only briefly with basic photophysics. An alternative approach, such as is in large part provided by Barltrop and Coyle's book, *Principles of Photochemistry,* should be welcomed by undergraduates and postgraduates alike. The emphasis of the book is on photophysics rather than photochemistry (consequently the title is inexact, for only in Chapter 6 is photochemistry really discussed).

After a brief introduction in Chapter 1, Chapter 2 describes time-independent excited-state properties. The Born–Oppenheimer approximation, Franck–Condon factors, spin-orbit coupling, $n\pi^*$, $\pi\pi^*$ and charge transfer transitions are covered. Also included are excited-state geometry changes, acid–base properties and solvent effects. Curiously, though, simple laser theory (which is dealt with too briefly, anyway) is also included in Chapter 2 rather than in Chapter 3, which deals with time-dependent properties, or in Chapter 5, in which techniques for examining reaction mechanisms are explained. Chapter 3 describes excited-state kinetics, fluorescence, phosphorescence, triplet–triplet annihilation and radiationless processes. The last topic is discussed simply but clearly although in a book of this type a more detailed discussion could have been included, especially considering the wealth of information now available. Quenching of excited states by excimer and exciplex formation, electron transfer and electronic energy transfer are treated clearly and concisely in Chapter 4. The kinetics of these quenching mechanisms are also detailed.

Methods of investigating reaction mechanisms, such as trapping intermediates, CIDNP or flash photolysis, occupy half of Chapter 5. No examples of transient flash spectra are given and the most important modern techniques of submicrosecond flash photolysis are only mentioned in passing. Similarly, measurement of singlet lifetimes is hardly mentioned except for an example in Chapter 3 of the rarely used two-photon fluorescence technique. The best feature of this chapter are the pages analysing quenching kinetics leading to nonlinear Stern–Volmer plots.

In summary, this book contains a great deal of useful and, on the whole, carefully presented information on the photophysical properties of excited states. Photochemical reactions are only briefly (12 pages) studied. The description of the experimental techniques is a little out-of-date and excludes important techniques such as single-photon counting and nanosecond and picosecond flash photolysis. The book is, however, well written and easy to read; the problems at the end of the book are also interesting and require some thought. The restriction of mathematical treatments to the minimum while retaining clarity should encourage less mathematically minded students to use this book. □

G.S. Beddard is Assistant Director of Research at the Davy Faraday Research Laboratory, Royal Institution, London, UK.

Atomic collisions

H. Kleinpoppen

Atomic and Molecular Collisions. By Harrie Massey. Pp.309. (Taylor and Francis: Basingstoke, UK, 1979.) £12.

AT LAST, one can say, there is an introductory authoritative text book available on atomic and molecular collisions. The author, Sir Harrie Massey, the grand old master and pioneer in the field, has written this introductory book shortly after the completion of the monumental and comprehensive monographs on atomic collisions (edited by him and his colleagues). The introductory book is suitable both for experts and non-experts in the field and particularly for undergraduate students. The book fills a gap and will certainly be welcomed by the scientific community.

The difficulties in preparing an introduction to atomic collisions mainly result from the nature of the subject. While introductory textbooks on solid-state physics or atomic spectroscopy, for example, can be restricted to the selection of stationary physical processes (which are dealt with by stationary quantum mechanical formalism), atomic collisions require dynamical methods and approximations over a wide range of applications. Even for the simplest colliding system such as scattering between electrons and atomic hydrogen no exact theoretical solutions are available. It was not possible to include sophisticated problems of atomic collision theory in an introductory text book. The book does contain a discussion and interpretation of the collision data required to develop basic concepts of quantum mechanics such as particles and waves and their dualism, the uncertainty principle, and the quantum mechanical description of scattering cross sections. Chapters on such topics include elementary descriptions of atomic and molecular structure. Appropriate experimental technology has been explained within the main text. Only a limited selection of topics on atomic collisions could be considered in this type of book. Nevertheless, the author has covered a broad range of the large field of atomic and molecular collisions and he has succeeded in exposing the full scope of the subject. It is obvious that the selection and detailed discussion of these most interesting topics reflect the mastery and long experience of the author as one of the pioneers in the field. The order of the topics of the book follows the traditional scheme, which is mainly guided by selecting projectiles and atomic and molecular targets: elastic and inelastic electron-atom scattering (including resonance effects, capture, recombination and attachment processes), collisions between neutral atomic and molecular systems, ion-atom collisions, photoionisation of atoms and molecules, and photodetachment of negative atomic and molecular ions. The last chapter deals with atomic collisions in the Earth's atmosphere, the solar corona and interplanetary space. Seven appendices supplement information mainly on experimental technology.

Each chapter, each section and even each line of the book is excellent, and it reveals the great devotion (and love) of Sir Harrie to the field of atomic collisions. The detailed discussions on selected topics (for example, the Ramsauer-Townsend effect, the glory and rainbow structure in atom-atom collisions, or the selected collision processes in the ionosphere) are of highest pedagogical standard. As an introduction the book represents without any doubt a break-through; it will be a challenge and precedent for future authors. This is the more important as the research field of atomic collisions is still in an explosive state: new exciting results from atomic angular correlations, from spin effects in electron-atom scattering, and from collisions with laser excited atoms will soon require new editions of introductory textbooks. Sir Harrie has paved the way (as he often did in research) for such future tasks. □

H. Kleinpoppen is Director of the Institute of Atomic Physics, University of Stirling, UK.

Nuclear physics

J.M. Reid

Elements of Nuclear Physics. By W.E. Burcham. Pp.422. (Longman: Harlow, UK, 1979.) £7.95.

THE accommodation of a rapidly expanding subject is a constant problem in the construction of undergraduate course syllabuses. The problem has been particularly severe in the case of nuclear physics over the past decade. The generally accepted separation of elementary particles (or high energy physics) as a subject in its own right has not produced any long-term amelioration in the face of the constant ferment of new ideas and approaches in both the low and high energy fields. Defined by the author of the volume under review as "the understanding of the nature of matter at a level deeper than that of the electronic structure of atoms, molecules and solids but not so deep as that of the particle structure of the proton and the neutron", nuclear physics must always find a place in some form in any course claiming to span matter from the macro to the micro level. The case for the study of the subject on the grounds of its practical applications has changed dramatically from the time of its emergence from the shadow of restricted work on nuclear based weapons in 1945. It was then, optimistically, to understand the processes about to provide a solution to the world's recognised energy problems. Today the case might be to provide a background understanding of the use of nuclear techniques in order to circumscribe the risks involved in the reluctant application of these techniques to socially vital problems.

However, Professor Burcham's latest textbook is not concerned with the practical applications of nuclear physics; it sets out to provide a description of the present state of nuclear structure measurements and to demonstrate what can be learned by the application of quantum mechanics to nuclear models. A previous well founded knowledge of atomic physics, relativity and quantum theory and some acquaintance with the rudiments of nuclear physics are required for maximum benefit to be obtained from this book, as the containment of the material within the limits of a single volume is achieved by very compressed treatment of matters assumed covered in earlier courses. Space is thus gained for a thorough treatment of the single particle shell model, the collective model and the unified model. The final three chapters in which the whole field is reviewed from the standpoint of the electromagnetic, the weak and the strong interactions are particularly valuable. A generous supply of numerical problems is included chapter by chapter and the solutions of a selected number of these are given in an appendix.

The final-year undergraduates for whom the book is designed will in no sense find this an easy text. Those among them who have postgraduate research ambitions will find it an excellent base on which their future studies in nuclear or elementary particle physics can be securely built. In fact for several years to come the text should be suitable reading for postgraduate courses in the field. The volume is put together with the neatness and the authority to be expected from an experienced and respected practitioner in this area of research. □

J.M. Reid is Professor of Natural Philosophy in the University of Glasgow, UK.

Applying electrodynamics to astrophysics

M. Rowan-Robinson

Theoretical Physics and Astrophysics. By V.L. Ginzburg. Pp.464. (Pergamon: Oxford, 1979.) Hardback £25; paperback £10.

PROFESSOR ter Haar has performed the arduous task of translating this advanced textbook by a prominent Russian astrophysicist admirably. Impressive though this book is in several respects, however, I feel it needed a good deal more work on it before it was published in a Student Edition.

A more accurate title for the book would have been *A Treatise on Electrodynamics, with some Applications to Astrophysics.* Most of the book consists of rather academic discourses on the themes of those great books by Landau and Lifschitz. However, Ginzburg does not have quite the same ability to illuminate abstract principles with simple and concrete examples. The absence of any sub-headings within the chapters makes the book hard to read and limits its usefulness as a work of reference. The neglect of the Western literature on the subject in the references cited (over 80% of the references are Russian and about a third are to papers by Ginzburg and coworkers) will not commend the book to other workers in the field, despite Ginzburg's plea in the Preface to the English edition that this was not so noticeable in the Russian edition and that the book was based on a lecture course. It is precisely this kind of deficiency in a lecture course that one expects to be made good in a textbook. The publishers have managed entirely to omit the first four pages of Chapter 3.

The most interesting and useful chapters for the general astrophysicist will probably be Chapter 5 on synchrotron radiation and Chapters 15–17 on cosmic-ray, X-ray and gamma-ray astronomy, though the review of the observational situation in these latter areas is rather out of date (the Russian edition appeared in 1974). Not all the formulae given are of sufficient accuracy for contemporary astrophysical applications. In the formulae for bremsstrahlung, for example, the Gaunt factor is assumed to be unity: some space could surely have been found for a discussion of the exact value.

The book remains, however, an important one by a major physicist. The specialist in electrodynamics will find it a useful review of Russian work in the field and the theorist in high-energy astrophysics will find some parts of the book valuable. □

Michael Rowan-Robinson is Reader in Astronomy at Queen Mary College, London, UK, and Gresham Professor of Astronomy at the City University.

Introducing meteorology

J.S.A. Green

Fundamentals of Meteorology. By L.J. Battan. Pp.321. (Prentice-Hall: Hemel Hempstead, UK, 1979.) £10.90. *The Atmosphere.* By Lutgen and Tarbuck. Pp.413. (Prentice-Hall: Hemel Hempstead, UK, 1979.) £10.90.

MOST of us have a vested interest in meteorology, and any books like those reviewed here, that seek to explain the intricate details of the subject to a wider audience, are always welcome. These two books summarise modern meteorology for the 'non-mathematician', by which the authors mean one not familiar with 'differential calculus'. Both books cover many topics of interest to a general reader, such as air pollution (supersonic airliners come in for substantial unquantitative criticism), clouds, the generation of rain, storms and climate. Curiously, their contents overlap by some 75%. To a scientist used to a uniform system of units, they both show a wide variety. One has a useful table of densities of common substances expressed in g cm^{-3}, as well as in kg m^{-3}. That the figures in one column are just 1,000 times the figures in the other might well strike the unitiated as an odd thing for scientists to pay attention to. A variety of basic units is used: calories, watts, feet, metres, knots, metres per second. Presumably the aim is to use units familiar to the reader, but one loses something of scientific nicety. The literary style is inexorable rather than objectionable: " . . . precipitation data for localities in selected precipitation regimes . . ." is typical.

My only major reservation concerns the sort of information that the authors have tried to present. A typical, but not unimportant, topic concerns the geostrophic wind. This blows along the lines of equal pressure, not across them from high to low pressure, as one might naively suppose. If I had to start from my first sight of Newton's laws of motion in order to arrive at this result, then I think I would feel cheated. The important fact is that the Coriolis acceleration is at right-angles to, and proportional to, the wind and not as one would expect, along the direction of the change in velocity as we usually measure it relative to the Earth. The point is made by having an experimenter, who used to be a golfer (but who now uses intercontinental ballistic missiles), fire missiles from the North Pole while the rest of the Earth moves past relative to the missile. But this rightly leaves me unimpressed because the golfer is in a situation where the real acceleration of the missile is zero, and the apparent acceleration seen from the Earth is fictitious. For geostrophic motion where the Coriolis acceleration balances the force due to the pressure field, it is exactly the other way round: the relative acceleration is small but the real (Coriolis) acceleration is large. Thus, trying to represent what really happens in terms that are too simple, may mislead that thoughtful non-mathematician. It might be better to tell him that geostrophic balance happens and that it is because of the rotation of the Earth. However, if you go and study mathematics, you can find the inevitable connection between the Newtonian laws and the geostrophic wind.

One expects to find authors representing such differences in philosophy that this topic highlights, and the books do summarise much modern meteorology, with particular emphasis on conditions in North America. Certainly one (but not both) would be a useful teaching aid for any non-meteorologist wishing to give a course on meteorology to American undergraduates. They are a useful *Introduction to Meteorology,* with the mathematical bits left out, and nicely illustrated by satellite pictures. To me they seemed to lack something of the enthusiasm of the authors for their subject. One of them recommends Minnaert's book, *Light and Colour in the Open Air,* to those interested in optics. Minnaert starts his book: "A lover of Nature responds to her phenomena as naturally as he breathes and lives." Now that is the sort of attitude I think we can expect to put before this person who has failed to acquire 'differential calculus' amongst his grades, and it does not come over to me from either of these texts.

Let us hope that their readers will absorb the philosophy and enthusiasm of Minnaert, as well as the useful factual information which he will find in these books. □

J.S.A. Green is Reader in Physical Climatology at Imperial College, University of London, UK, and Head of a Group teaching meteorology at MSc. level to students with a first degree in mathematics or physics.

Principles of applied geology

John Knill

Principles of Applied Geophysics. By D.S. Parasnis. Pp. 275. (Chapman and Hall: London, UK, 1979.) Hardback £8.50; paperback £4.95. *Geology for Civil Engineers.* By A.C. McLean and C.D. Gribble. Pp. 310. (Allen and Unwin: Hemel Hempstead, UK, 1979.) Paperback £5.95.

THESE two books, while illustrating quite different aspects of industrially orientated geology, have also somewhat different status as books. Parasnis' book is the third edition of a well known volume which has been reprinted three times between editions, while McLean and Gribble have written a brand new text.

Geophysics has been for many years the major large-scale exploration tool applied to hydrocarbon search, is also widely used in the mineral and construction industries and is now of increasing significance in ocean and land-based shallow crustal studies based on artificial explosions. This text has an extremely systematic approach, separate chapters dealing with magnetic, gravitational, electrical and seismic methods with additional chapters on induced polarisation, electromagnetic methods, radioactivity techniques and miscellaneous methods including borehole logging. The popularity of this book as a brief but comprehensive survey of the field of technique, processing and interpretation in applied geophysics is well justified. The new edition includes a new chapter on induced polarisation, derivation of formulae has been put into appendices to aid the flow of the text and the units and symbolism have been standardised and clarified. This book will undoubtedly continue to fulfill a need for an inexpensive but comprehensive presentation ideal for the student at undergraduate and postgraduate level.

The textbook by McLean and Gribble is primarily directed at the undergraduate civil engineer and provides a basic introduction to geology, and to those aspects of applied geology relevant to the construction industry. The book has a fresh, attractive appearance and the cover is particularly striking, tending to draw the eye, and possibly the wallet. The authors have thought carefully about the philosophy of the book but, in the event, selected the traditional balance between geology and engineering geology on an equal basis. The early chapters review minerals and rocks, engineering soils, stratigraphy and structural geology. The contents are deliberately selective, focusing on those terms believed by the authors to be of the greatest importance to the engineer. The book then turns to, and deals with, ground and surface water, site investigation including geophysics, rock classification and materials, excavations, slope stability, dams and reservoirs, and tunnels. The chapters on engineering geology draw upon several case histories which are briefly illustrated. There are many clear diagrams and photographs, and each chapter is supported by references. The authors have clearly made a careful attempt to cover the field but there are gaps, some of which are important. For example, there is no reference to soft ground boring techniques, which is surprising after the detailed consideration given to engineering soils, and foundations appear to be wholly omitted. The authors have drawn mainly upon their own experiences in developing the book and occasionally the text jars to the expert mind. A little more honing of the text in parts would have helped because, to the reviewer, occasional sections or phrases were inappropriate in relation to either conventional practice or the conclusions drawn. However, there is no doubt that the audience for whom the book has been written will find it easy to read, interesting and possibly stimulating. □

John Knill is Professor of Engineering Geology and Head of the Department of Geology at Imperial College, London, UK.

Chemical ecology

P.E. Howse

Introduction to Chemical Ecology. By M. Barbier. Translated by M. Ferenczi. Pp.128. (Longman: London and New York, 1979.) £3.95.

MICHEL BARBIER, a natural products chemist, attempts in this book to review our current knowledge of toxins, defensive secretions, plant allomones and sterols, and to define chemical ecology as a discipline.

My first criticism concerns Mr Ferenczi's translation, which does a disservice to the author. He frequently fails to find the appropriate English idiom, resulting in absurdly tortuous expression as, for example, in the quotation in the paragraph below. His evident lack of familiarity with entomology leads to the common silkworm (*Bombyx mori*) being described several times as a "butterfly" or as the "mulberry tree Bombyx". Worse still, the gypsy moth is described as "the predatory forest butterfly", and the boll weevil as the "predatory cotton plant beetle". It is unfortunate that the publishers did not, apparently, check the translation for syntax, idioms, or use of common names. There are also many minor errors that should have been picked up in proof.

My second argument is with Barbier's concept of chemical ecology, to which he devotes much of two chapters, a preface and an epilogue. He defines chemical ecology as "the science of chemical relationships between living organisms or between living organisms and the mineral world". This allows him to include pollution problems and aspects of nutrient cycling in the same ambit with animal secretions, hormones and allelochemicals, and to suggest that the whole can lead to a "new philosophy of man" which predicts "the absolute necessity of changing rapidly man's psychology" (that is, in his approach to environmental problems). He (and Ferenczi) make some rather trite observations in terms that render them almost incomprehensible: ". . . death is a primary condition of life . . . to develop life is akin to increase in death potentials, including various forms of pollution. In this instance, the contradiction is not thought to be fundamental, but rather to be the mirror image of a progress vector". This kind of writing will discourage and confuse any reader, but I firmly believe that such philosophical flights are unnecessary anyway.

Chemical ecology is surely a search to explain interrelationships between organisms and their environment in chemical terms, rather than in terms of traditional ecological factors like climate, niche, food resources, and so on. Chemical ecology is now at a very exciting stage: we now have a good fund of information on the chemistry of animal secretions used in communication and defence, secondary plant substances, and the like. We know very little about the adaptive function of these, but that information is beginning to accrue and the general principles for which Barbier is casting around will be found in the field in coming years. Ecology in general has been concerned for many years with pollution and its effects on natural equilibria, but the methodologies, concepts and supporting disciplines are distinct from those involved in explaining interrelationships between, say a heliconid butterfly, its food plants and its predators, in terms of chemical factors. □

P.E. Howse is in the Chemical Entomology Unit at the University of Southampton, UK.

Ecology booklets

P. D. Moore

Island Ecology. By M. Gorman. Pp.79. (Chapman and Hall: Andover, UK, 1979.) £1.95. *Vegetation Dynamics.* By J. Miles. Pp.79. (Chapman and Hall: Andover, UK, 1979.) £1.95.

THE days are gone when undergraduate texts adequately embraced broad subjects. The development of a whole range of specialist studies even within a field like ecology has rendered it difficult for any one author to deal effectively with the rapidly growing body of information and ideas. Publishers are facing this problem in one of two main ways; either they are turning to edited textbooks in which each chapter has a different author (for example, *Thoretical Ecology* edited by R.M. May (Blackwell: Oxford, 1978)) or they resort to part-works in which various specialists are responsible for brief booklets which are intended to fit together into a uniform whole. This approach has been most effectively pioneered by Edward Arnold in their Institute of Biology series, which now runs to over a hundred items.

Chapman and Hall are now entering this market by producing an ecological series designed on similar lines and edited by George Dunnet and Charles Gimmingham at Aberdeen. The first two issues in this series are reviewed here.

Vegetation Dynamics by John Miles, of ITE (Institute of Terrestial Ecology), Banchory, is essentially a study in plant succession and it represents a very thorough and up to date review of this area of ecology. It begins by considering the ways in which ecologists view vegetation, with emphasis upon the divergence in viewpoints since Gleason and Clements. Mechanisms of vegetation change are dealt with fairly briefly, which is an unenviable task since such wide subjects as seed dormancy, competition and allelopathy need to be covered in a single chapter. This is not entirely satisfactory, since the subject warrants a book on its own and much has to be conveyed by examples, and no example can ever fully typify an ecological generalisation. In particular, more space could have been given to a consideration of stability, its definition and conceptual value. As it is, one is left with an uncritical statement of Horn's logical, but somewhat unhelpful, definition relating stability to speed of return to the equilibrium state.

A consideration of cyclic changes concentrates upon the well known A.S. Watt examples, plus regeneration of *Abies* forest. It would have been sensible to develop this idea more fully in its application to the persistence of the climax state. The regeneration mosaic of the climax, as demonstrated by such workers as Loucks, Forcier and Hope-Jones, provides an interesting comparison with the cyclic phenomena described in such detail by Watt.

There is finally a consideration of the role of grazing animals, another subject in which generalisation is difficult. Perhaps its influence on population dynamics of plant species could have received more emphasis.

One area which is rather neglected in the booklet is the total ecosystem view of succession, but perhaps the title was chosen specifically to avoid this issue. Nevertheless, a more complete work would have resulted if the Odum analysis of succession (which is mentioned) had been critically discussed. Such subjects as energy flow and nutrient cycling are central to an understanding of succession.

Overall, I wish the booklet could have been a little longer to include these additional items. Within the spatial confines of 80 pages, Miles has produced an extraordinarily extensive review of over 300 papers and has collated them in an interesting and thought-provoking manner. I shall certainly direct students towards it.

Island Ecology by Martyn Gorman is concerned with a very much more confined topic than vegetation dynamics and thus is more easily dealt with inside the restricted space available. The subject is again one which is of interest to many ecologists because of the manner in which the biogeographical principles propounded by McArthur and Wilson have been applied generally to fragmented habitats at a variety of scales.

The bulk of this booklet is concerned with true islands and their biology, but the idea of 'habitat islands' is always held in mind. For example, when considering dispersal, reference is made to B.N.K. Davis' experiments with nettle clumps and their invasion by invertebrates.

The mathematics of island biogeography is dealt with in a clear and simple manner and will provide a useful starting point for introducing students to this concept. The evolutionary significance of islands is also considered. The final two chapters are devoted to habitat fragmentation, both on the scale of mountain ranges and at the nature reserve level.

The bibliography for this topic is, not surprisingly, considerably smaller than that for vegetation dynamics. The editors should have insisted upon a uniformity of arrangement of references which, in this book, are placed at the end of chapters.

This new series has commenced with two useful and readable booklets and subsequent titles must be awaited in hopeful anticipation. Some of the future authors, we gather, will derive from areas other than eastern Scotland; the home team has set them a high standard to maintain. ☐

P.D. Moore is Senior Lecturer in the Department of Plant Sciences at King's College, University of London, UK.

Microbial ecology

P. S. Meadows

Microbial Ecology: A Conceptual Approach. Edited by J.M. Lynch and N.J. Poole. (Blackwell Scientific: Oxford, 1979.) Hardback £19.50; paperback £9.80.

THIS book is a mixture of styles and subjects, and standards — some of them excellent, a few mediocre and one or two very poor. The book is divided into three major sections on (1) the principles of microbial behaviour in ecosystems, (2) microorganisms in their natural environments, and (3) economic microbial ecology.

Much of the first of these is interesting reading, although I am not convinced of the need for a detailed description of the range of microorganisms (chapter 2.1). The mathematical treatment of population and community dynamics in chapter 2.3 is not easy to follow unless one has a good grasp of mathematics. It could have been presented in a simpler way without loss of much detail.

The second section contains the main part of the book with six chapters. Chapters on the terrestrial and aquatic environment are followed by the animal as an environment, extreme environments, aerial dispersal and biological interactions. Parts of these are well presented and interesting; but there are some omissions and a few inadequacies. Much of the chapter on the aquatic environment is to my mind too general, and is not strictly related to microbial ecology except in a very broad sense. For example, the large diagram on the major oceanic gyres is not really necessary. Biological interactions (chapter 3.6) contains two pages on the rumen and caecum (a field that would not be regarded as ecological by some ecological purists), whereas the relationships between microorganisms and invertebrates in sediments and on solid surfaces in aquatic environments receives hardly any comment.

The last major section — on economic microbial ecology — has one first-rate chapter on nitrogen fixation, two chapters of variable standard on microbial spoilage of food and on water pollution, and one quite extraordinary one on recalcitrant molecules.

The book has excellent parts to it.

However, I have three major criticisms. The first stems from the book being a compilation of chapters by different authors. This has led to fragmentation, but not to an unacceptable degree. The second is more serious. Some of the diagrams are impossible to understand except after long contemplation (Figs 3.2.6, 3.2.17, 3.5.8, 3.5.12), which is a pity because some are models of clarity (Figs 2.2.12, 3.2.15). The third criticism is one about writing English. Parts of the book are badly written and this can only reflect on the authors. The introduction and epilogue and especially the chapter on recalcitrant molecules should not have been published in their present form. They are often long-winded, difficult to understand, and grammatically wrong.

All of this is a pity because many other parts of the book are very readable and interesting. □

P. S. Meadows is a Senior Lecturer in Zoology at the University of Glasgow, UK.

Microbial physiology and nitrogen fixation

C.F. Thurston

Bacterial Metabolism. By G. Gottschalk. Pp.281. (Springer: New York, Heidelberg and Berlin, 1979.) $23.00. *The Biology of Nitrogen-fixing Organisms.* By J.I. Sprent. Pp.196. (McGraw-Hill: Maidenhead, UK, 1979.) £5.50. *Microbial Physiology.* By A.G. Moat. Pp.600. (Wiley: New York and Chichester, UK, 1979.) £16.95.

THESE books provide an intriguing study of the different approaches that can be taken when writing for students. All three are concerned more or less with metabolic processes of microorganisms, but there is little else in which they are similar.

Bacterial Metabolism, by G. Gottschalk, is accurately described by its title. It is about the biochemical transformations achieved by prokaryotic organisms and in my opinion is the best concise treatment of the subject available. The text is clear and well illustrated with metabolic schemes and much tabulated information. It starts with a chapter on bacterial nutritional requirements and types which is followed by two large chapters: "How *Escherichia coli* synthesises ATP during aerobic growth on glucose" and "Biosynthesis of *Escherichia coli* cells from glucose". These chapters set a framework which is built on in subsequent chapters to form a very complete picture of bacterial metabolic activity, showing the variations exploited when different substrates for growth are used and/or when different organisms are involved. For instance, the descriptions of catabolic activities of aerobic heterotrophs and of chemolithotropic modes of metabolism are more thorough and detailed than in any other comparable student text book I have seen. Of the topics included, protein biosynthesis has the least generous coverage; but as this is so well described in many modern textbooks of molecular biology and/or general biochemistry its coverage here is sufficient. All chapters have an informative summary and there is a list of titles for further reading, and subject indexes. The last chapter concerns the fixation of molecular nitrogen, which is dealt with from a biochemical standpoint, consistent with the rest of the contents of this readable and informative book.

Nitrogen fixation in the broader context is the subject of *The Biology of Nitrogen-fixing Organisms*, by J.I. Sprent. The very wide range of prokaryotes which are able to fix gaseous nitrogen are described with something of their classification. This is integrated with descriptions of the other organisms with which many nitrogen fixers are involved in elaborate symbiotic relationships, although in the case of nitrogen-fixing lichens this treatment is brief. The second chapter examines the biochemistry of nitrogen fixation, including properties of nitrogenase, sources of energy and reductant and some mechanisms which allow nitrogen fixers to reduce or prevent the oxygen inhibition to which nitrogenases are very sensitive. This chapter is not always easy to follow and is illustrated with diagrams which lack clarity, for instance in Figs 2, 3, which show the photosynthetic electron transport chain of *Anabaena*, it is obscure as to where reducing equivalents originate. I was also most disappointed that there was no inclusion of the genetics of nitrogen fixation. The conventional genetic analysis of the *nif* genes of *Klebsiella pneumoniae* and the more recent studies using the techniques of 'genetic engineering' at Sussex and Harvard have contributed much towards the understanding of nitrogen fixation and for many people (whether rightly or wrongly) suggest a possible way for dramatically improving the productivity of world agriculture. I think it was wrong to omit this work altogether.

The following three chapters discuss nitrogen fixation in relation to agriculture, forestry and natural ecosystems. They are an absorbing account of research in plant physiology and experimental ecology presenting much original data showing the importance of fixation to the nitrogen economy of different ecosystems, and the considerable challenge presented to biologists wanting to understand what limits the quantity of nitrogen fixed in the field and what might increase the amount of fixation. The final chapter describes possible evolutionary relationships of nitrogen-fixing organisms and is followed by an informative appendix describing methods of measuring nitrogen fixation. The text is well supported with references, as many as 100 for some chapters.

Microbial Physiology, by A.G. Moat, also uses specific references throughout the text which are listed at the end of each chapter, but otherwise it has little similarity with Dr Sprent's book. According to its dust cover the book draws together the current knowledge of the fine structure (cell structure, substructure), metabolism, and genetics of microorganisms and describes the interaction of these physiological activities in the growth of microbial cells. . ." I find this approach to what constitutes the physiological activities of microorganisms curious and I am confident that others will be equally surprised to find the first 120 pages of a book with this title devoted to classification and cell structure. From Professor Moat's preface it emerges that this is substantially a course book for his students and reflects an interesting and broad ranging course in general microbiology, albeit with a physiological emphasis. It is also directed particularly at "students who will ultimately choose to work outside the immediate realm of microbial physiology". I think other microbiology teachers may find the choice of material and the relative depth of coverage not well suited for their students.

The book is well illustrated and contains a lot of detailed useful information, but classification, cell structure, microbial genetics and morphogenesis together occupy over a third of the text. Consequently, I feel it is unfortunate in a book of this size and price that some truly physiological topics are omitted and others are given relatively scant attention. For instance, growth is the subject of one chapter, but the relationship between limiting-nutrient concentration and growth rate is not described; continuous (chemostat) culture is mentioned only twice, in passing, is not defined and is illustrated by a schematic diagram (Fig. 9-5) where the growth chamber contents would regularly siphon out — it is in fact a recycling fed-batch culture apparatus! Parameters such as growth yield and maintenance energy are not mentioned and macromolecular turnover gets little attention. There are substantial chapters on carbohydrate metabolism and energy production, on nitrogen metabolism and on amino acids, purines and pyrimidines, but I was unable to find a description of any mechanism for nutrient uptake or how such a process may be driven at the expense of metabolic energy. I think I can accept that a course in microbial physiology does not have to include the *lac* operon of *E. coli* as an example; but I deplore the absence from a textbook of the subject, of any description of the control system of a well studied inducible operon, such as *lac, ara, gal* or *hut*. I was also surprised to find only occasional mention of catabolite repression, with in one instance, a hint that this might be a cyclic-AMP mediated phenomenon; indeed the unqualified and unsupported statement that "the enzymes of the glyoxylate bypass are repressed . . . by a process known as catabolite repression" infers a broader definition of this phenomenon than I have encountered elsewhere. Another area of confusion concerns the assimilation of carbon dioxide by phototrophic microorganisms. First, the text states "Photoautotrophs . . . fix carbon dioxide via either the reductive pentose phosphate (Calvin–Benson) pathway or the reductive C_4-dicarboxylic acid (Hatch–Slack) pathway. These systems were first discovered in green plants". I would infer that the unicellular green alga *Chlorella* (with which Calvin's group worked) is regarded by the author as a green plant and not a microorganism, were *Chlorella* not specifically mentioned as a group of organisms included in the eukaryotic protists (page 12). Second, Fig. 3-21 entitled "The reductive C_4-carboxylic acid (Hatch–Slack) cycle" is actually the reductive (or reverse) tricarboxylic acid cycle proposed by Evans, Buchanan and Arnon, which is dependent on two ferredoxin-catalysed carboxylation reactions characteristic of anaerobic metabolic systems and which are not present in the higher plants which show the C_4 pattern of assimilation investigated by Hatch and Slack. Indeed the role of phosphopyruvate as a product of CO_2 fixation in the reverse TCA cycle contrasts strongly with CO_2 fixation by C_4 plants where phosphopyruvate is generally considered to be the co-substrate for the key carboxylation reaction.

The reader will by now conclude rightly that I would not recommend A.G. Moat's book, and there are several general microbiology texts I would prefer.

Returning briefly to the other two volumes discussed I am pleased to have read them, I shall return to them again and I think they both represent good monetary value. *The Biology of Nitrogen-fixing Organisms* has an engaging scholarly style and I am sure will be widely read by biology students and others. Gottschalk's *Bacterial Metabolism* is excellent and I will be surprised if it is not extremely popular amongst students and teachers of microbiology and biochemistry for a very long time. □

C.F. Thurston is a Lecturer in the Department of Microbiology at Queen Elizabeth College, London, UK.

Microorganisms in their environment

J.P. Armitage

Microorganisms: Form, Function and Environment. Edited by L.E. Hawker and A.H. Linton. Second edition. Pp.391. (Edward Arnold: London, 1979.) Paperback £8.95.

THE second edition of this undergraduate textbook has changed format. It is now a fashionable large size book with large, bold print and diagrams. Its sixteen chapters, with twenty-two authors (all, except one, from the University of Bristol), have been divided into three sections: biochemistry and physiology, form, size and life cycles of microorganisms, and the activities of microorganisms in their environment.

The strength of the first edition lay in the good mix of prokaryotic and eukaryotic microbiology and the emphasis on their behaviour in the environment. To this end this edition has added an excellent new chapter on symbiotic interactions.

However, the new format has resulted in many fewer words than the first edition and, rather than keeping or expanding the better areas of the first edition, all sections have been reduced. This results in much weaker coverage of many areas, in particular biochemistry.

The editing has also been hit and miss: for example, *Klebsiella pneumoniae* is referred to as *K. aerogenes* in the first section, but by its correct name in the second; *Rhodospirillum* has been misspelt and page references to other parts of the book have been left incomplete.

The difficulty with all multi-author textbooks is keeping the level and theme consistent. Unfortunately, by reducing the size of this book but attempting to retain all the subject matter of the first edition the result is a weaker textbook with little to recommend it over the other general textbooks on the market. □

J.P. Armitage is a Postdoctoral Research Scientist in Microbiology in the Department of Botany and Microbiology, University College London, UK.

Soil properties

Stephen Nortcliff

Geography and Soil Properties. By A.F. Pitty. Pp.287. (Methuen & Co Ltd: Andover, 1979.) Hardback £10; paperback £5.50. *Soil Erosion.* By R.P.C. Morgan. Pp.113. (Longman: Harlow, UK, 1979.) Paperback £3.95.

NOTWITHSTANDING the title of this text, the major shortfall of the first book is a failure to place the wealth of information on soil properties into a geographical context. The opening chapter presents a wide ranging review of soils and geography, both physical and human, together with a brief comment on soils and geographical method. In subsequent chapters little attention is paid to geography; rather, the emphasis is placed on detailed descriptions of numerous soil properties.

Although the detailed information presented together with an extensive bibliography of over 40 pages is evidence of considerable effort in compilation, frequently much of the information is presented without a context; also it is not shown why so much detail is important to the geographer. At times less information coupled with more elaboration and discussion would have produced a more complete and easily readable text more suitable for undergraduate use.

The second book is one of a series of short texts in applied geography suitable for undergraduates studying in the fields of environmental sciences, geography or geology. In a little over one hundred pages the author has set himself the task of making the reader aware of the importance of soil erosion as a problem throughout the world, the mechanics and processes of soil erosion, the factors which influence soil erosion, soil conservation practices and the scope for modelling soil erosion studies, as well as giving some examples of soil erosion studies.

Remarkably the author achieves this, but only by presenting the reader with just a brief outline of each topic. Any additional information must be added by the reader, and to this end there is a bibliography chiefly of erosion studies. As an undergraduate text this approach may be acceptable, but at times a little elaboration or development of the topic would have greatly improved the text. In the introduction the author stresses that although soil erosion has long been acknowledged as a problem in tropical and semi-arid areas, it is increasingly recognised as a hazard in temperate countries. It is a pity that few of the examples used refer to erosion in these temperate countries. □

Stephen Nortcliff is Lecturer in Soil Science at the University of Reading, with teaching responsibilities in pedology and soil management.

Plant systematics and taxonomy

V.H. Heywood

Plant Classification. By L. Benson. Second edition. Pp.901. (D.C. Heath and Company: Lexington, Massachusetts and Toronto, 1979.)

In the 22 years that have elapsed since the publication of the first edition of this work, plant systematics and taxonomy have experienced major changes, but these are scarcely reflected in this new edition. It is intended as an elementary textbook "to open up the new world of living plants to college students and the educated public". The author refers readers to his *Plant Taxonomy, Methods and Principles* (Ronald: New York, 1962, 1978) for an advanced treatise covering the application of cytogenetic and experimental investigations to plant taxonomy.

On the other hand, new information and ideas on the origin and relationships of the major groups of vascular plants other than the flowering plants are taken into account in the appropriate sections of this edition. For the flowering plants, four new systems of classification, namely those of Stebbins, Takhtajan, Cronquist and Thorne, are considered (chapter 16), though not fully accepted; and the author has presented a revised version of his own system, the most conspicuous change being the final abandonment of the Amentiferae as a partly natural, partly artificial group. No mention is made of other recent systems such as those of Dahlgren and Soó. The section of the book devoted to the classification of natural floras, primarily with reference to North America, has also been revised.

Overall, this new edition of Benson's major work retains the merits and faults of the original. It is too long (nearly 900 pages) and diffuse and covers too many diverse topics for a single work. It is an excellent book to dip into, but is scarcely likely to recommend itself to students outside North America as a textbook of systematics, although it contains a large amount of information that would be useful for various courses. The book is well printed and illustrated, but the binding in my review copy has already come adrift. □

V.H. Heywood is Professor and Head of the Department of Botany, University of Reading, UK, and currently Dean of the Faculty of Science.

Fungal biology

P.E. Long

Biology of the Fungi: Their Development, Regulation and Associations. By Ian K. Ross. Pp.499. (McGraw-Hill: New York and London, 1979.) £11.20.

Professor Ross sets out to provide an introductory undergraduate text emphasising developmental and regulatory aspects and has succeeded by presenting, in a conversational style, a lively, questioning and personal account both of the fungi and of the protistan mycetozoa and their allies traditionally studied by mycologists. Some will find, as the author anticipates, that treatments of particular groups or topics offend their susceptibilities but a reasonable balance and wide coverage have been achieved in the three sections that deal with the salient features and developmental pathways of the organisms under consideration; discuss the regulation of their vegetative and reproductive development; and review their associations. The latter, shortest section includes a welcome chapter on viruses in fungi but is really a rather loose mixture, strictly fungal, additionally ranging from their spore liberation and dispersal through saprobic, mutualistic, parasitic and predatory activities to their impact on human affairs.

My initial enthusiasm became tempered by the impression that the survival strategies of the septate higher fungi and their capacity to produce varied multihypal structures are not sufficiently described. Furthermore the roles of vacuolation and septal pore occlusion in thallus development are neglected as is much experimental work both on conidiation and the cell cycle in yeasts, while hyphal wall architecture and synthesis receive rather cursory attention. To be fair many of these deficiencies can be rectified by diligent use of the extensive bibliography although this remedy is not available for readers of chapters 15 and 16, as, atypically for this up-to-date textbook, few post-1970 books on parasitism or mutualism are cited. Some more generally useful sources are overlooked too, including Ainsworth and Bisby's *Dictionary of the Fungi* (Sixth edition, Commonwealth Mycological Institute, 1971). The figures, mainly in the first section, are good but many lack any indication of scale and some captions are so terse as to be uninformative. Hopefully the appellation 'death cup' that appears under Figure 6.36 will not become perpetuated.

These expressions of vexation denote my exasperation at points which diminished the appeal of a stimulating and attractive book that provides many ideas and much material for a modern course in mycology. The approach is fresh, the arguments enjoyable and some topics are new in a text which will be particularly useful to students who have had some previous exposure to university level cell biology, while also providing food for thought to their instructors. □

P.E. Long is a Lecturer in Mycology in the Department of Botany at the University of Leicester, UK.

Introductory soil science

D.L. Rowell

Introduction to the Principles and Practice of Soil Science. By R.E. White. Pp.198. (Blackwell Scientific: Oxford, 1979.) £8.50.

Although there have been several books published recently which deal with soils from a geographical viewpoint, this is the first book of its kind to appear for many years. It is a valuable attempt to present soil science at an introductory level, and has successfully shown how biology, chemistry and physics are integrated in the study of this part of our environment. It is thoroughly up to date, including for the first time at this level much that has been understood only recently.

The first two sections deal with the principles of soil science and the third part looks at the application of these principles to soil management, primarily from an agricultural point of view. The book is suitable for use in a wide range of courses at universities, technical and agricultural colleges. It assumes O level knowledge of the sciences, but occasionally starts at a higher level — surely a mistake for a book of this sort. Material is presented very effectively in diagrammatic form. The text is sometimes very concise, perhaps making it difficult for the book to be used in isolation from taught courses. □

D.L. Rowell is Lecturer responsible for the teaching of introductory soil science courses in the Department of Soil Science at the University of Reading, UK.

How plants work

James Sutcliffe

Plant Physiology. By R. G. S. Bidwell. Second edition. Pp.726. (Collier-Macmillan: London and Basingstoke; Macmillan: New York, 1979.) £14.25; $18.95 in USA. *Organization in Plants.* By W. M. M. Baron. Third edition. Pp.264. (Edward Arnold: London; University Park Press: Baltimore, 1979.) Paperback £6.75.*Physiology of Woody Plants.* By P. J. Kramer and T.T. Kozlowski. Pp.811. (Academic: New York and London, 1979.) $35; £22.75. *Plant Metabolism.* By G. Richter. Pp.475. (Croom Helm: London, 1979.) £11.95. *Plant Metabolism.* By H. D. Kumar and H. N. Singh. Pp.302. (Macmillan: London and Basingstoke, 1979) Paperback £4.95. *The Control of Growth and Differentiation in Plants.* By P. F. Wareing and I. D. J. Phillips. Second edition. Pp.347. (Pergamon: Oxford, 1979.) Hardback £15; paperback £7. *Water Flow in Plants.* By J. A. Milburn. Pp.225. (Longman: London and New York, 1979.) Paperback £6.75.

FOR those of my generation who studied plant physiology during and immediately after the second World War choice of textbooks was extremely limited. Now the situation has changed dramatically and students in the 1980s have a wide range of attractive texts some of the more successful of which have recently appeared in new editions. Comparison of a lavishly illustrated modern textbook such as Bidwell's *Plant Physiology* with those that were available to me in the 1940s shows how much the standards of book production have improved in the intervening years.

A popular successor to James' *An Introduction to Plant Physiology* as an introductory text is Baron's *Organization in Plants.* First published in 1963 the book has been extensively revised for the third edition. Like his predecessor, Baron places great emphasis on the encouragement of practical work and it is good to see graphs summarising data obtained in class experiments illustrating the text. An appendix gives details of a number of experimental procedures which should be well within the scope of most school laboratories, and a useful starting point for student projects. Some advice about the design of experiments and simple statistical procedures for the analysis of results would be a welcome addition to this section. A new chemical nomenclature, which I assume is now being taught in schools, is used throughout the book. I suppose that we shall all get used to it in time, but is it really wise to call acetaldehyde ethanal which can be so easily confused with ethanol, however logical it might seem to be? It is something of a relief to be informed that acetic acid for some unexplained reason is still acceptable to the powers that be.

Nowadays a problem for writers of plant physiology textbooks is the lack of general botanical knowledge among their readers because the teaching of traditional botany in schools has almost ceased. To overcome this, Baron provides some background information about the structure of plant cells, but for vital details of plant anatomy the student must go elsewhere. It is a pity that the list of books for further reading at the end of chapter 1 does not include a textbook of general anatomy.

Bidwell's *Plant Physiology,* the first edition of which appeared in 1974, is one of several excellent textbooks by North American authors which give comprehensive coverage of the whole field of plant physiology at a fairly advanced level. Although like most other contemporary texts it concentrates mainly on the physiology of flowering plants with particular reference to those crop plants which have been studied intensively, there is a section devoted to the physiology of what are described as "special" organisms. These include trees, as one might expect from a Canadian-based author, macroscopic algae, parasites and symbionts. In keeping with modern trends space is found for such topics as the effects of stress on plant growth and the influence of man on his environment.

Students who wish to learn more about the physiology of trees should turn to Kramer and Kozlowski's *Physiology of Woody Plants.* This book is an amalgamation and up-dating of two earlier books by the same authors. It is written primarily for foresters and arborists but inevitably it contains a good deal of general physiology. Biochemical topics are reviewed rather briefly and the book concentrates on more physiological and ecological aspects of plant physiology. There are useful chapters on the structure of wood and the characteristics of tree growth. A final chapter is concerned with the influence of environmental factors and cultural procedures on the productivity of forests and orchard crops. The text is supported by a large amount of experimental data and in addition to a substantial list of general references at the end of each chapter there is a bibliography of some 2,200 titles.

Plant biochemistry has been the subject of several new textbooks recently, among them Gerhard Richer's *Plant Metabolism.* This is an English (Americanised) translation of an apparently successful German textbook, *Stoffwechsel Physiologie der Pflanzen,* which was first published in 1969. Although the text has been revised for the English edition the more recent references quoted are to papers published in 1973 so that in certain areas of this rapidly advancing field the book is already somewhat out-of-date. More than a third of the volume is taken up by an account of photosynthesis which is well presented with the help of clear informative diagrams. The remainder of the book deals with such topics as respiration, the biosynthesis of cellular components and metabolic regulation in a straightforward and fairly conventional manner. No attempt is made to relate metabolic processes to growth but there is a brief incursion into plant water and ion relations and a section is devoted to the role of anions and cations in metabolism.

Extensive use is made of small print when describing experimental procedures and historical aspects of the subject. This enables the author to get much more information into a book of modest dimensions than would otherwise have been possible. However, this and the liberal use of italics throughout the text give the book a rather outdated look which I do not feel will appeal to American readers for whom it is apparently intended.

At less than half the cost of Richter's book one may obtain Kumar and Singh's *Plant Metabolism* which covers very similar ground at about the same level. The new edition of a book which was first published in India in 1976 has been printed there and does not reach the high standard of book production that we have come to expect from the publishers. Again quite a large part of the text is concerned with photosynthesis and as both authors have worked on unicellular algae it is not surprising that algal research features prominently in their account; but there is an adequate treatment of the various pathways of carbon assimilation in higher plants too. The rest of the book is less than inspired, being in the main a rather pedestrian account of the metabolism of nitrogen and sulphur, nucleic acids, proteins, lipids and secondary plant products. In the preface, the authors urge their readers to consult original papers but they provide very little incentive by failing to capture in their writing much of the excitement of biochemical research. Their rather unselective suggestions for further reading including, for example, several outdated textbooks and reviews, are not particularly helpful.

A large part of plant physiology is concerned with the processes of growth and development and there are several textbooks devoted entirely to this subject. One of the most popular of these in recent years has been Wareing and Phillips' *The Control of Growth and Differentiation in Plants* which was first published in 1970. The book is mainly concerned with the effects of plant growth regulating substances on various facets of plant growth. In the second edition the material has been re-organised and parts of the text have been revised in the light of developments in the intervening years. A comparison of the two editions indicates that apart from advances in understanding of the role of phytochrome in photomorphogenesis, which now merits a separate chapter, and the control of flowering there have been no outstanding discoveries in this field during the 1970's. It must have been somewhat depressing for the authors to find that so many of their pious hopes of advances expressed in 1970 remain unfulfilled in 1978. I think that in the second edition I would have felt inclined to alter the form of words a little and explain why certain problems have remained surprisingly intractable. The picture is not perhaps quite so black as Wareing and Phillips have made it appear because they do less than justice to the not-inconsiderable advances that have been made in the intervening years in knowledge of control mechanisms at the enzyme level. Fortunately there are other books which remedy this deficiency.

One of the most difficult subjects to put over to students of plant physiology is water relations. Teaching this subject is not helped by the less than adequate treatment of it in many textbooks. Authors of such books would do well to read Milburn's stimulating book on *Water Flow in Plants* before revising this section of their works. Milburn begins by describing the properties of water and he gives a clear account of the concept of water potential before going on to describe various kinds of apparatus by means of which water movement can be studied. He then describes the water relations of cells and traces the pathways of movement through the soil-plant-air continuum. There is an interesting chapter on responses to water stress and a description of a new device "the oil bomb" which may prove helpful for studying effects of stress on growth.

There is a useful set of appendices containing lists of symbols, units, physical data etc., and a series of numerical problems which will exercise the minds of some university teachers and tutors as well as their students. ☐

James Sutcliffe is Professor of Plant Physiology in the University of Sussex, Brighton, UK.

Vegetation productivity

Melvin G.R. Cannell

Vegetation Productivity. By G. Jones. Pp.100. (Longman: Harlow, UK, 1979.) Paperback £3.95.

THIS little book is an attempt by a geographer to introduce people without any knowledge of plant science to the subject of vegetation productivity in different environments and the ways in which that productivity can be measured. The book is easy to read and may satisfy geography students, but it adds nothing to the books already available on this subject.

Chapter 1 is a introduction to plant physiology, misleadingly entitled "The measurement of plant growth". None of the concepts of plant community productivity are discussed, and photosynthesis itself gets only a brief mention. Chapters 2 and 3 on vegetation productivity contain useful reviews, but readers wanting accurate, balanced and more comprehensive accounts will need to refer to Cooper's book (*Photosynthesis and Productivity in Different Environments.* International Biological Programme 3, Cambridge University Press, 1975) which is not mentioned. Also, anyone seriously interested in measuring forest or grassland productivities will need the handbooks prepared by Newbould (*Methods for Estimating the Primary Production of Forests.* International Biological Programme, Handbook No. 2, Blackwell: Oxford, 1967) and Milner and Hughes (*Methods for the Measurement of Primary Production of Grassland.* International Biological Programme Handbook No. 6, Blackwell: Oxford, 1968) which form the bases of chapters 4 and 6, respectively. Chapter 5 describes the author's work on forest growth in relation to soils, and chapter 7 offers some conclusions.

It is a pity that this book was not co-authored or edited by a plant scientist: vegetation is said to "produce" calories (page 14), transpiration is confused with respiration (page 19), a linear regression is said to follow a curve (page 55), and so on. Many figures and tables are poorly explained and both metric and imperial units are used. Furthermore, the thesis of the book, that a natural plant community provides a measure of the upper limit of vegetation productivity in a given environment, is, at best, highly contentious. ☐

Melvin G.R. Cannell is Principal Scientific Officer and Tree Crop Physiologist at the Institute of Terrestrial Ecology, Penicuik, UK.

Plant diseases

I.M. Smith

Epidemiology and Plant Disease Management. By J.C. Zadoks and R.D. Schein. Pp.427. (Oxford University Press: Oxford and New York, 1979.) £13.50.
Atlas and Manual of Plant Pathology. By E.H. Barnes. Pp.325. (Plenum: New York and London, 1979.) £12.35.

IT is a pleasure to welcome Zadoks' and Schein's textbook. Plant disease epidemiology has developed enormously in theory and practice in the last twenty years, and the ideas of management in plant protection are spreading from pests to diseases. No university plant pathology course can now fail to tackle these new developments. This new text, though possibly not quite a primer for students with only basic knowledge, provides an excellent introduction to the subject for advanced students. As the authors suggest, the student can choose to benefit from any of the attractive self-contained chapters. They will at all times find clarity of exposition and an interesting and engaging style.

The work builds from the basic level of single infection cycles (monocyclic processes), through population phenomena (polycyclic processes), to the whole pathosystem and its management. It builds from the basic methods of disease control to their integration, from the familiar to the less familiar. It is realistic and critical, and pleasantly free from mysterious jargon.

The core chapter on the essential concepts of epidemiology and their mathematical background is clearly developed and well illustrated, presenting few problems to the not very mathematical reader. There is an excellent chapter on disease and crop loss assessment. Chapters on "Ecological concepts in plant pathology" and "Matters of scale" bridge the sections, the former providing useful comparisons with general ecological concepts.

The text does not make any attempt to deal with the techniques or apparatus of epidemiology, which are perhaps somewhat beyond its scope. However, practical considerations are well in evidence, especially in the assessment chapter, and a most useful feature is the inclusion of simple argued examples from the literature. Many chapters conclude with a useful set of exercises. In conclusion, this is an excellent reasonably priced textbook which will certainly find very wide use in advanced courses in plant pathology for years to come.

The *Atlas and Manual of Plant Pathology* is, by comparison, not a work of any outstanding interest. A republication of a 1968 edition, with minor modifications, it treats a series of diseases on a taxonomic basis, giving general information on each (causal organism, symptoms, control), together with black and white photographs at macroscopic, hand-lens and microscopic levels. The descriptions are sometimes supplemented by details of laboratory experiments, which are possibly the most interesting part of the work and have not especially dated since the first edition. However, epidemiology receives little attention. The numerous photographs are certainly useful for the systematic study of plant diseases, However, with a certain prejudice against prepared slides, the present reviewer regrets the non-inclusion of any instructions on how to examine fresh or cleared material. At the other end of the scale, a modern text should surely show some of the insights given by electron microscopy.

The text is also regrettably out of date, and indeed misleading in many respects. Nomenclature is sometimes outdated and recent developments bypassed (crown gall). Most unfortunate perhaps is the very old-fashioned section on plant virology. The point is that, in this and many other respects, plant pathologists can be quite proud of the progress made in the last decade. It is a pity, therefore, to republish an old textbook, without taking the trouble to revise it. ☐

I.M. Smith is Deputy Director-General of the European and Mediterranean Plant Protection Organization, Paris, France.

Plant breeding

Barbara Pickersgill

Principles of Crop Improvement. By N.W. Simmonds. Pp.408. (Longman: Harlow, UK, 1979.) £7.95.

THIS is the first new textbook on plant breeding for about 12 years, but a decade is a short time to a breeder so the subject matter discussed remains basically unaltered. Where this book differs from its predecessors is in its excellent treatment of conservation of genetic resources and the green revolution and, more particularly, in its uncompromisingly practical orientation.

Background information on crop evolution, population genetics, polygenic inheritance and heritability is used to discuss the breeder's very real problems of breeding strategy, selection, scale of operations and profitability in either social or real terms. Academically elegant special cases such as aneuploidy in barley or regulation of recombination in wheat breeding get little mention. Indeed, cytology takes a back seat throughout. Tropical crops provide about as many examples as temperate crops, which should increase this textbook's appeal to those teaching in developing countries.

Professor Simmonds writes clearly and concisely but two stylistic traits detract slightly from the book's readability. One is the intentional use of very long captions to tables or figures. By the time one has assimilated those, one tends to have lost the drift of the textual exposition the table or figure was to illustrate. The other is the use of abbreviations. Sentences such as "Thus, summarising the slightly confusing nomenclature of OPP breeding, we have: PIM (varieties propagable as such) contrasted with SYN (varieties regularly reconstructed from parents), with the former based on either MSL or PRT" may completely baffle students who find even basic genetic terminology difficult. Unfortunately there is no glossary, nor are abbreviations listed as such in the index.

Numerous thought-provoking examples for the reader to work are embedded in the text or captions and carry the textual argument further. It is therefore advisable to do one's homework thoroughly before assigning class reading in this book.

On price, this text compares favourably with most of its competitors. It seems likely to achieve its author's hope of providing a broad general introduction to plant breeding as an intensely practical art. ☐

Barbara Pickersgill is Lecturer in the Department of Agricultural Botany at the University of Reading, UK.

The natural selection

M.J. Lawrence

Introduction to Evolution. By F.A. Racle. Pp.161. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) £5.80. *The Biological Environment.* Edited by J. Lenihan and W.W. Fletcher. Pp.164. (Blackie: Glasgow and London, 1979.) Hardback £9.95; paperback £4.95. *Evolution.* By C. Patterson. Pp.197. (British Museum (Natural History)/ Cornell University Press: London, 1979.) Hardback £5.95; paperback £2.95. *Evolving: The Theory and Process of Organic Evolution.* By F.J. Ayala and J.W. Valentine. Pp.452. (Benjamin/ Cummings: Menlo Park, California, 1979.) $16.95. *An Introduction to Evolutionary Genetics.* By D.T. Parkin. Pp.220. (Edward Arnold: London, 1979.) Paperback £6.50. *Evolution.* Readings from Scientific American. Pp.135. (Freedman: San Francisco and Reading, UK, 1979.) Hardback £7; paperback £3.20. *Ecological Genetics.* By E.B. Ford. Pp.442. (Chapman and Hall: London, 1979.) Paperback £7.50.

THIS is a very mixed batch of books which vary greatly in the breadth and depth of their treatment of evolution and related subjects, in their intended readership and in their length. Some, having been written for the layman, assume no previous knowledge of the subject, while others require at least a knowledge of elementary genetics and biology and are, therefore, of a more specialised interest.

Racle's book, though one of the shortest, attempts to cover the full range of evolutionary studies. Because it is intended for undergraduate non-science majors in American universities and colleges, it contains chapters on elementary geology, which is treated in an historical fashion, and Mendelian genetics. The chapter on population genetics is the least satisfactory not least because it is not at once made clear that the Hardy–Weinberg equilibrium applies only to a population of randomly mating individuals. Discussion of the sickle-cell polymorphism would also have been better done in this chapter rather than in the preceding one dealing with Mendelian genetics. The rest of the book treats the subject in an orthodox fashion and there are chapters on the evidence for evolution and natural selection, on the origin of life, phylogenetic divergence in the plant and animal kingdom, and two chapters on human evolution, though surprisingly, there is little about molecular evolution. The wide coverage of the subject in this short book, which is written in a pleasantly undemanding style, would make it suitable for use in schools.

Lenihan and Fletcher's book is the ninth volume in a series on *Environment and Man* and is intended for "practitioners and students in science, engineering, medicine, administration and planning". It is a rather odd and uneven book with few illustrations or diagrams. The chapters by Fletcher on "Evolution and the Natural Environment", by Ainsworth Harrison on

"Human Evolution and the Environment", by Lenihan on "Is Man a Machine?" and by Smith on "Microbes and the Environment" are straightforward and well written. Jeffers' chapter on "Conservation of the Biological Environment" is authoritative and full of relevant information, though perhaps a little less easy to read than the others. Forbes Robertson's chapter on "Natural Selection in Man" is far too demanding for the intended readership and is, frankly, rather dull. I also have some doubts about the wisdom of attempting to produce a series of books that is aimed at such a wide, semi-professional readership, though with some guidance, this particular volume could also be used in schools.

Patterson's book is a very well written, lucid and up-to-date account of the subject, which ranges from molecular biology to the philosophy of science. Though a short book, it contains numerous excellent illustrations, one of which, on Batesian mimicry in butterflies, is in high-quality colour. The fate of new mutations in a population is particularly well handled. It is to be regretted, however, that the explanation of the concept of fitness is done in terms of gametic rather than the more usual zygotic genotypes (page 58). Fortunately, this has no unfortunate consequences because thereafter the author very sensibly deals with changes in gene frequency in the population in purely verbal terms. Though the book is intended for the layman, its excellence is such that it would be suitable for use both in the sixth form and in the early years of undergraduate courses in biology in universities.

As one would expect from a pair of experienced authors and researchers in the field, Ayala and Valentine's book is a very broad, competent, well illustrated and up-to-date treatment of evolutionary ideas and studies, which, like Racle's book, has been written with the collegiate layman in mind. However, because of its length, the authors are able to deal with the subject in much greater depth than in any of the preceding books. For this reason, the book would serve as an excellent introduction to the subject for the non-specialist undergraduate biologist also. Any teacher recommending the book for this purpose, however, would be obliged to point out a quite unforgivable error in diagrams on pages 31 and 32 which show that the chromosomes are double structures in the early stages of the first division of meiosis, whereas, of course, they are in fact single until late in the pachytene stage. This is such a serious error that it ought to be corrected by an erratum slip at least before any further copies of the book are sold.

Parkin's book has been written for undergraduate students in the biological sciences and assumes, therefore, some knowledge of Mendelian and cytogenetics. Unfortunately, it is a rather uneven book. The first chapter on evolution by natural selection, which gives a sketch of the historical background, is nicely written as are some parts of most of the following chapters. In far too many places, however, the point that the author is trying to make is not very clear, which is partly a consequence of his having included too many poorly digested examples. There are also a number of errors. For example, the probability of fixation of a neutral mutant is $1/2N$, not $1/2N_e$ (page 34); East and Mangelsdorf, rather than Emerson, were the first to describe the genetics of self-incompatibility in the system found in *Oenothera organensis* (page 103); and heritability is not the proportion of the genetic variance that is due to additive genetic variance (page 114). In addition, several of the figures are either hard to understand or hard to relate to the text (for example, Figs 6.3, 6.5, 7.6 and 8.7). There are also more than a small number of misprints and typographical errors. It is a great pity that the book is marred by these errors for it would otherwise be a useful addition to the literature on evolutionary genetics at this level.

The nine chapters in the *Scientific American* book, which originally appeared in the September 1978 number of this journal, cover the full range of the subject from chemical evolution and the origin of life (Dickerson) to the evolution of man (Washburn). There is an introduction by Mayr and the remaining chapters are by Ayala on "The Mechanism of Evolution", by Schopf on "The Evolution of the Earliest Cells", by Valentine on "The Evolution of Multicellular Plants and Animals", by May on "The Evolution of Ecological Systems", by Maynard Smith on "The Evolution of Behaviour" and by Lewontin on "Adaptation". Though the articles have presumably been written with the layman in mind, their authority and the excellence of their illustrations are such that they are, in my view, a better introduction to modern ideas about evolution for the intelligent biology student than any of the preceding volumes.

I have deliberately left the best book of the batch until last, for Professor Ford's book is a classic and is nearer to the original literature on the subject than any of the others. Its inclusion in the list arises solely from the fact that it has now most sensibly been issued in paperback form which should make it more accessible to both undergraduate and graduate students in the biological sciences. Though not everybody will agree with all that Professor Ford says, for the book is very much a personal view, he, together with Dobzhansky (and not only the latter, as Ayala and Valentine would have us suppose), have surely contributed more to our understanding of selection in natural populations than any other two men. □

M.J. Lawrence is Senior Lecturer in the Department of Genetics at the University of Birmingham, UK.

Genetic variability

Alan E.H. Emery

Laboratory Manual of Genetics. By A. M. Winchester. Pp.148. (C. Brown; Dubuque, Iowa, 1979.) $5.95. *Investigating Chromosomes.* By A. F. Dyer. Pp.138. (Edward Arnold: London, 1979.) Paperback £6.75. *Understanding Genetics.* Second edition. By N. V. Rothwell. Pp.682. (Oxford University Press: Oxford, 1979.) £14. *The Elements of Genetics.* By I. H. Herskowitz. Pp.442. (Collier-Macmillan: West Drayton, UK, 1979.) *Human Genetics: A Selection of Insights.* Edited by W. J. Schull and R. Chakraborty. Pp.361. (Dowden, Hutchinson and Ross: Stroudsburg, Pennsylvania, 1979) $30. *Human Genetics: Problems and Approaches.* By F. Vogel and A. G. Motulsky. Pp.700. (Springer: Berlin, Heidelberg and New York, 1979.) DM98; $53.90.

A NUMBER of textbooks of genetics have been published this year. But they differ so much in approach and content that it is not possible either to compare them or to present them under any unified theme.

Two essentially practical guides which I found appealing are A. M. Winchester's *Laboratory Manual of Genetics* and A. F. Dyer's *Investigating Chromosomes.* Winchester's book is a truly laboratory manual with practical information on such topics as the making of *Drosophila* culture media and the preparation of root tip mitoses (though not animal mitoses). Much of the book, however, is devoted to answering problems relevant to material given in the text. There are work sheets for answers but no solutions are given. It embraces both plant and animal genetics and there are useful sections on human genetics including the preparation of karyotypes from photomicrographs of stained chromosome spreads. For a first course in general genetics this laboratory manual could be most valuable.

Dyer's book is also very practical but at a more advanced level and is mainly concerned with the cytogenetics of flowering plants. The structure and behaviour of chromosomes are studied using essentially one basic preparative technique which is described in detail. It is well illustrated with simple line drawings and photomicrographs, and details are provided of the chromosome constitution of many flowering species. Each chapter concludes with a list of references for further reading. This book will be of particular value in departments of botany.

Turning now to student textbooks of general genetics we have N. V. Rothwell's *Understanding Genetics* and I. H. Herskowitz's *The Elements of Genetics*. Both authors have had considerable experience in teaching genetics over the years and this is reflected in the clarity of their respective texts. Rothwell's book is perhaps slightly more advanced. Examples are chosen from a wide variety of organisms from phage to man. The sections on molecular genetics are excellent. It is extremely well illustrated with line drawings and each chapter concludes with up-to-date references and review questions, answers to which, teachers will be relieved to hear, are provided at the end of the book. There is also a very good glossary. How this book will fair in the face of many competitors will only be decided by students and teachers who work through it. Certainly it is a very attractive textbook.

Herskowitz's book is basically a simplified version of his *Principles of Genetics* (Collier-Macmillan: West Drayton, UK; second edition, 1977) which I reviewed in *Nature* two years ago and concluded that this is one of the best undergraduate textbooks of general genetics currently available. But whereas the *Principles of Genetics* is essentially a complete textbook covering the entire field and suitable for a one-year course, the present text will fulfill the needs of a one-term course. For this purpose I have no reservations at all in recommending it. It is very up-to-date and there is also sufficient material on human genetics for those in health science courses. Each chapter concludes with a summary and questions (for which answers are provided), and references for further reading. There is also a glossary of terms. It deserves to be successful.

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Finally, two recently published books on human genetics merit special consideration. The first consists of a selection of 26 classic papers in human genetics with comments by the editors, W. J. Schull and R. Chakraborty. This is Volume 10 in a series of *Benchmark Papers in Genetics*. In his Foreword, the series editor, David Jameson, states that papers have been selected which "demonstrate both the development of knowledge and the atmosphere in which that knowledge was developed. There is no substitute for reading great papers". There is little doubt that students should be encouraged to read the original papers in which major contributions to a subject were made. The papers in the present volume are arranged into three sections. The first section is largely historical and deals with the formal genetics of man with selections from such as Garrod, Bateson, Farabee, Landsteiner, Morton, Edwards and Haldane. The second section is concerned with the relationship between genes and chromosomes and human disease, and the selections here include papers by such as Lejeune, Carr, Pauling, Levine and Penrose. The third section is concerned with the nature and maintenance of genetic variability in man and includes papers by Haldane, E. B. Ford, Hirschfeld, Allison and Harry Harris. The editors contribute a final chapter on some of the social, ethical and legal problems which have been precipitated by recent developments in human genetics.

It is interesting to compare this selection with a similar one edited by S. H. Boyer in 1963 *(Papers on Human Genetics,* Prentice-Hall: Englewood Cliffs, New Jersey). Inevitably, and despite protests by the editors of such volumes, the selection of papers for inclusion will reflect, to a greater or lesser extent, the particular interests of the editor himself. Thus, Boyer's book leans more toward biochemical genetics, and Schull and Chakraborty's book toward population genetics. Interestingly the authors of these two books agree on only four papers: Garrod on alkaptonuria and chemical individuality; Pauling on sickle cell anaemia as a molecular disease; Allison on protection from malaria afforded by sickle cell anaemia; and Lejeune on the chromosome constitution of mongolism. I would therefore recommend both books to any would-be student, though in both he will find little in the area of clinical genetics, for example seminal papers by Carter, Clarke, Lenz and McKusick. Also I think I would take issue with Schull and Chakraborty's stated intention to ". . . distinguish between those creative acts with no apparent, immediate antecedents and those, which like the specification of the genetic code, may have been creative from the laboratory viewpoint, but represent the culmination of an intellectual ferment so pervasive as to dominate the activities of numerous laboratories. . . . surely a contribution of the former kind does not lie outside the prospects of any gifted scientist whereas the latter too frequently depends on accoutrements rather than insight". I would contend that in science the former is in fact rare and that almost all developments, including those in human genetics, are the result of selection, reshuffling, combining and synthesising of already existing facts, ideas, faculties, and skills. This is of course elegantly argued by Koestler in his *The Act of Creation.* Nevertheless this is an excellent collection of papers and the editors' comments are informative, interesting and germane. It can be highly recommended.

The second book on human genetics is by F. Vogel and A. G. Motulsky, whose expressed aim has been to produce a book which is ". . . a fairly thorough and up-to-date treatise on the conceptual basis of the entire field of human genetics and its practical applications". In this they have been completely successful. It is as thorough and precise as Vogel's earlier text *(Lehrbuch der Allgemeinen Humangenetik,* Springer, 1961) but is much more modern in outlook with less on anthropology and mathematical genetics. The current text is comprehensive and covers all aspects of human genetics and, as would be expected of authors as internationally regarded as these two, it is authoritative. It is illustrated by 420 very clear figures, and there are over 1,700 references provided for further reading. There is both an author index as well as an extensive subject index, and there are nine appendices into which are collected some of the more mathematical treatments of individual subjects. Despite its thoroughness, however, clinical aspects of the subject are largely ignored. Nevertheless, this is likely to become the standard postgraduate textbook concerned with the science of human genetics for many years to come. It is an excellent book and can be highly recommended. ☐

Alan E. H. Emery is Professor of Human Genetics at the University of Edinburgh, UK.

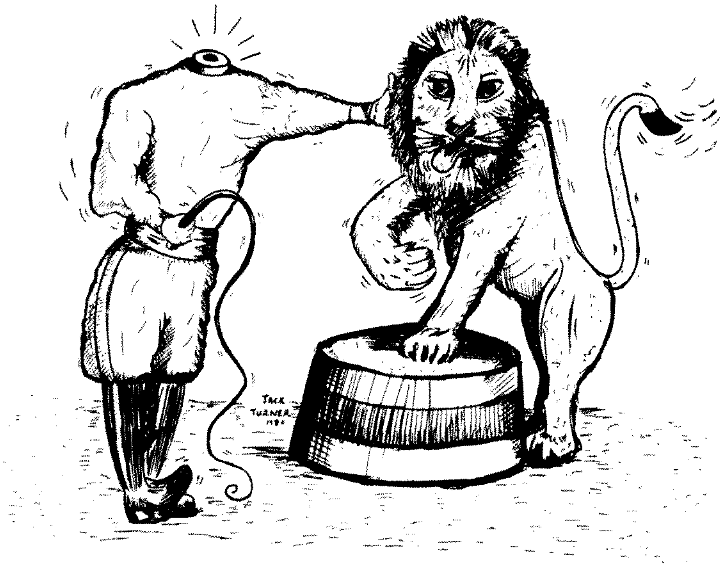
Managing wildlife

T.H. Clutton-Brock

Wildlife Management. By R.H. Giles. Pp.416. (Freeman: San Francisco and Reading, 1979.) £11.40.

As human populations grow and plant and animal populations shrink, it becomes increasingly important for the former to control and manage the latter. The pressing need for trained managers has produced an eruption of wildlife management courses and Giles' book provides an introductory text for this growing market. The book includes chapters on the analysis and manipulation of animal populations, on habitat description and design, on controlling the effects of human populations and on the problems of managing particular groups of animals, from fish to bears.

Wildlife Management is written in an easy, colloquial style, and provides many clearly worked examples of numerical techniques and a list of study questions at the end of each chapter. It has more than its share of facile statements ("Wildlife management is a decision science; an extinct animal is one lacking survivors; all living populations are dynamic"), luxuriant verbiage ("People are an integral part of the population-habitat-people triad of wildlife management (see Figure 1-1)") and unnecessary jargon ("feedforward" for shaping the future of a system; "ecological amplitude" for the range of conditions in which an animal can survive; "opportunity cost" for the compensation that people require to forego behaviour which might harm wildlife). And to an English reader, a proportion of the section headings are ambiguous: for example, it was not immediately clear to me that sections on *Alpha-Person Theory* and *Waterfowl Violations* would deal with analyses of who breaks game laws and the frequency with which legislation involving waterfowl is infringed.



"Don't worry, it'll be all right on the night."

Just what is wildlife is not, according to Giles, very clear. Game species are definitely wildlife but wild flowers, lichens, viruses and nematodes are not. Butterflies may or may not be and fish are sometimes excluded (apparently because of the wiles of a US fisheries commissioner who persuaded Congress to change the name of the US Biological Survey to the US Fish and Wildlife Service, thus providing his particular branch of science with a place in the sun but consigning fish to an ecological purgatory somewhere between birds and invertebrates). This somewhat arbitrary selection, Giles argues, is necessary because "there is a danger of opening a conceptual umbrella so wide that it covers all biology . . .".

But surely this is precisely what wildlife management should aim to do. It is unfair to blame Giles for the implied dichotomy between wildlife biology and ecology since this is common in the wildlife literature. However, the attitude is a dangerous one, especially in an introductory text, for it could cause wildlife managers to believe that they do not require a close acquaintance with ecological and biological theory. This is far from the case and ignorance of fundamental principles can only produce mistaken management policies. For example, a better knowledge of modern developments in ethological and evolutionary theory would surely have led Giles to modify his views that disease may have evolved as a density-regulating mechanism in some species and can be 'healthy' for such populations; that there is very little evidence for the inheritance of behavioural traits; and that inbreeding seldom has deleterious effects (pages 38 and 75). Such misconceptions could well have a dangerous effect on management policies. Moreover, only a broad understanding of ecological theory will allow managers to predict how previously unmanaged populations are likely to react to novel policies. Wildlife management is not an art form evolving into a separate science, as Giles suggests in his opening chapter. It is one branch of ecology and will only suffer if it is separated from other areas of biology. ☐

T.H. Clutton-Brock is at the King's College Research Centre, Cambridge, UK.

Adaptation and regulation

D. L. Ingram

Principles of Animal Physiology. By J. A. Wilson. Second edition. Pp.891. (Collier-Macmillan: West Drayton, UK, 1979.) £16.45; $30.20. *Animal Physiology. Adaptation and Environment.* By K. Schmidt-Nielsen. Pp.560. Second edition. (Cambridge University Press: Cambridge and New York, 1979.) Hardback £22.50; paperback £6.95. *Adaptation to Thermal Environment.* By L. E. Mount. Pp.333. (Edward Arnold: London, 1979.) Hardback £16; paperback £7.50.

THESE three books all share the common themes of adaptation and regulation; but the scope of each is quite different.

Principles of Animal Physiology is a comprehensive textbook covering the whole field of animal physiology, both vertebrate and invertebrate, in nearly 900 pages. It is written entirely by one author and the fact that it is in its second edition must mean that it has been well received. The central theme with which the author has attempted to weld this wide variety of topics together is regulation, an idea which is in accord with the modern view of physiological systems seen in terms of engineering and mathematical models. A book by a single author has the advantage of the same style throughout; but the subject covered here is so great that it would be very surprising indeed if all the facets were covered with a great depth of understanding.

Just as writing on such a wide range of subjects has its difficulties so does reviewing this type of book, and those chapters which are close to my own subject inevitably attracted the greatest attention. There are places in the sections on energy exchange and thermoregulation where I feel that the specialised knowledge which can only be acquired by close contact with the subject is lacking and there are occasional errors. The student who comes new to the subject, however, is presented with material which is free from numerous qualifying statements and will learn the more subtle points at a later stage in his study.

The book begins with a section on the properties of living systems in which organisation at the cellular level is covered. In the second section the regulatory systems such as the contractility of cells, the nervous system and hormones are described. Finally, there is a section in which nutrition, thermoregulation, circulation, and water and solute regulation receive attention. There is also a good bibliography. Each chapter is very well illustrated with diagrams and figures, some of which have been taken from published work and some of which are the author's own. Numerous tables provide the student with a wealth of information and there are several charts presenting biochemical pathways and structural formulae of physiologically active compounds. The book is directed chiefly at the American market and would certainly provide enough material for a general degree in physiological zoology. The hard-pressed student will find all the topics covered in his course in a single book and this will have obvious attractions: but the more advanced student will need to consult other texts. The author has not attempted to cover mammalian physiology in the detail which would be required in courses for a medical or veterinary degree for which there are already a good selection of books.

Animal Physiology: Adaptation and Environment, also in its second edition, is a really excellent book, covering as its title suggests a particular aspect of physiology. The author himself describes the book as elementary, and although it is true that the background required is minimal, it would be a mistake to regard this as just an elementary text. There will be very few experienced physiologists who will not gain a greater understanding of their subject from this account. Many topics which may seem difficult in other books are presented here in such an easy manner that the reader has understood the point before he realises there could be any problem. The wide range of research and discussion in which the author has engaged in various places all over the world has provided him with an understanding that ensures that just the right examples are always used to illustrate a point.

The book does not, however, make any claim to being a complete textbook of animal physiology, and although both invertebrates and vertebrates receive attention, the subject matter is intentionally selective. Physiological systems are dealt with in five parts. The first is headed oxygen and deals with respiration in water and air, and the transport of gases in the body. The second is concerned with food and energy. The third and fourth parts cover temperature, and osmotic regulation. In the final part there is a discussion of movement, integration and the way animals deal with information. The text is well illustrated and there is a good selection of references. I think that this book will serve two functions, one as an introduction which will raise the student's interest, and the other as an account for advanced students who want to organise the mass of facts which they have acquired into an understanding of principles.

Adaptation to Thermal Environment: Man and his Productive Animals is in many respects like an expanded second edition of the author's earlier successful book which was concerned with the climatic physiology of the pig, and is now out of print. This book is much more specialised than the other two reviewed here, being devoted entirely to the thermal aspects of the environment. This general topic has been the subject of several specialised meetings and as in many other branches of physiology considerable progress has been made during the past three decades. The author gives a well presented and highly factual account of the field with particular emphasis on the quantitative aspects of the subject and includes over 500 references to reviews and original papers.

In the first half of the book the nature of heat exchange between the animal and its environment receives very competent treatment and the physical aspects are presented in a way that should be understandable to students without a detailed knowledge of physics or mathematics. Many of the tables and figures are of use not only as illustrations of the text, but also as a valuable source of reference. In the second half the author deals with the various sorts of environment which animals encounter and concentrates in particular on the reactions of those species which are of economic importance, as well as to man himself.

Students of applied biology will find this book of special value because a great deal of information has been collected from a wide range of sources and presented in a digested form. Although the subject matter is specialised, the book is not an advanced text and is suitable for students with an elementary knowledge of biology and physics. I feel that its particular success lies in demonstrating the ways in which the reactions of animals to their environment are governed by the rules of physics. For this reason it may well find a readership outside the usual scope of biology amongst engineers and designers. ☐

D. L. Ingram is Senior Principal Scientific Officer at the ARC Institute of Animal Physiology, Babraham, Cambridge, UK.

All you need to know about proteins

C.C.F. Blake

Principles of Protein Structure. By G. E. Schulz and R. H. Schirmer. Pp. 314. (Springer: New York, Berlin and Heidelberg, 1979.) £13.95.

It is an extraordinary fact, often taken for granted, that just one class of molecule, the proteins, is responsible for *all* the active functional aspects of biological organisms, and for much of their structure as well. Whether one considers catalysis, metabolite transport and storage, molecular defence, blood-clotting, hormonal control, gene expression and muscle contraction; or bone, tendon, membranes and cytosol: in all these processes proteins are the central, active principle. Luckily this vast diversity of protein function is not paralleled by an equivalent diversity of protein structure: in fact the principles of protein structure are remarkably simple. The past 20 years have seen an absolute avalanche of detailed information on the structure of proteins which now provides a firm foundation for understanding the systematics of protein structure and function.

Schulz and Schirmer's *Principles of Protein Structure* is the first comprehensive attempt to assemble the knowledge gathered over these last two decades. The first part of the book is broadly analytic. Starting with the types of amino acids and the structural implications of the peptide bond, Schulz and Schirmer take us through the covalent and non-covalent forces that determine the patterns of folding and association of polypeptide chains. There then follow chapters on the prediction of secondary structure; thermodynamics and kinetics of chain folding; protein-ligand interactions; and a final chapter on the structural basis of protein function. This second part is broadly synthetic, attempting to use the information from the first part to understand the folding of proteins and the acquisition of function. Unfortunately this logical scheme is interrupted by two oddly positioned chapters on representation of structure and on protein evolution. That the authors feel some unease at the organisation of the book is indicated by their injunction to begin at chapter 7! That is scarcely necessary; chapter 1 is a good enough starting point, but new readers should be warned that chapters 7 and 9 are misplaced.

Having disposed of this minor criticism, I can welcome this book with wholehearted admiration. The topic could so easily have led to a diffuse descriptive text, whereas what is presented is analytical and tightly argued. Each chapter is multiply subdivided which makes for easy reference. Lest anyone be concerned about the command of language of two German authors, Schulz and Schirmer write in a lucid and, at times, elegant English that is never less than a pleasure to read. All in all a totally recommendable book. □

C. C. F. Blake is Lecturer in Molecular Biophysics at the University of Oxford, UK.

Welcome enzymology

G. Lowe

Enzymatic Reaction Mechanisms. By C. Walsh. Pp.978. (Freeman: San Francisco and Reading, 1979.) £18.30.

Enzymology is a subject which has relevance to and devotees from a wide range of disciplines. In this most welcome addition to a growing number of texts the author's approach is based on the chemistry of the reactions catalysed. In this way he has collated the transformations of metabolites in biological systems into a relatively few types of chemical reaction.

The book is in five main sections. In Section I the chemistry of the reactions to be dealt with in the remainder of the book is considered and general comments made on enzyme specificity and catalysis. Section II is concerned with enzyme catalysed group transfer reactions. Acyl-transfer being the most thoroughly studied is considered first. Unfortunately a highly significant paper concerning the catalytic triad of amino acid residues in the serine proteases appeared too late to influence the discussion in the text, but was incorporated as a footnote. This is a nightmare any author faces who attempts to survey a rapidly developing subject.

For a textbook that is devoted to enzymic reaction mechanisms, it is refreshingly free from those algebraic expressions which make enzyme kinetics look so formidable. There is however a very useful account of the application of kinetic isotope effects and reversible inhibition of enzyme catalysed reactions. Glutamyl- and amino-transfer reactions are the subject of the next chapter and this is appropriately followed by two chapters on phosphoryl transfer which includes discussions on phosphatases, ATPases, phosphodiesterases and kinases. This group of enzymes has, however, been the focus of a good deal of attention in the last few years, and inevitably therefore some highly significant experiments concerning the mechanism of these enzymes do not appear. Nucleotidyl and pyrophosphoryl, and glycosyl transfer reactions form the remaining chapters of this section.

Section III which consists of seven chapters focuses on enzymes and their cofactors which catalyse oxidations and reductions. Enzymes using nicotinamide, flavin and pterin co-enzymes are discussed first and this is followed by metallo-enzymes which utilise copper or iron as the redox component in catalysis. Finally molecular oxygen as an oxidising agent is discussed in which one (mono-oxygenases) or both oxygen atoms (dioxygenases) are used.

Section IV is concerned with enzyme-catalysed eliminations, isomerisations and rearrangements. A general theme is less evident in this section. Eliminations and isomerisation are however usually general acid–general base catalysed reactions, whilst rearrangements, which are fairly rare in enzymology, are often dependent on co-enzyme B_{12}.

Section V covers enzymic reactions that make and break carbon-carbon bonds, so bringing together some of the most biosynthetically versatile reactions, and which frequently require cofactors, such as thiamine pyrophosphate, biotin and pyridoxal phosphate. In the final chapter of this section the chemical logic of metabolic pathways is discussed.

The author is to be congratulated on producing an exciting and very readable account of the chemistry of enzymatic reaction mechanisms. The presentation is excellent, and the text is remarkably free from trivial error. □

G. Lowe is in the Dyson Perrins Laboratory, University of Oxford, UK.

Scope and applications of enzymology

Keith F. Tipton

The Nature of Enzymology. By R.L. Foster. Pp.383. (Croom Helm: London, 1979.) Hardback £19.95; paperback £9.95.

THIS book was an ambitious undertaking in that the author has attempted to survey the properties and function of enzymes and the medical, analytical and technological applications of enzymology at a level that would be suitable for undergraduates reading biochemistry and related subjects such as medicine, pharmacy, and applied biology. In trying to cover such a wide field it is, perhaps, not surprising that some topics are treated in scant detail; for example, there is no treatment of the kinetic analysis of enzyme reactions involving more than one substrate, the treatments of allosteric and cooperative effects are not sufficiently detailed and lack correlation with structural data; and the treatment of enzyme-linked immunoassays is too brief to explain the approaches adequately. Many of these deficiencies are, however, ameliorated in part by the well chosen references to original papers and reviews.

The mathematical analysis of enzyme behaviour is the weakest aspect of this book and the section on reversible inhibitors contains some confusing errors. In some places, such as the section on the regulation of enzyme systems, the purpose of the mathematical treatments is not made clear.

The strength of this book lies in its treatment of the more applied aspects of the subject, and the sections on the applications and limitations of enzymological methods in medicine and industry are clearly presented and well illustrated with examples. The use of numerous examples to illustrate the points made throughout the book are most valuable and make it enjoyable to read. Because of its somewhat cursory treatments of some important aspects of enzymology I could not recommend this as a textbook for students reading biochemistry as their main subject. Despite the criticisms mentioned above, however, the book might be used advantageously for courses in which biochemistry was a peripheral subject and it could be recommended to anyone wishing to get an idea of the present scope and applications of enzymology. □

Keith F. Tipton is Professor of Biochemistry at Trinity College, Dublin, Eire.

Biochemistry texts

E.D. Saggerson

Elementary Biochemistry: An Introduction to the Chemistry of Living Cells. By Julian Davies and Barbara Shaffer Littlewood. Pp.346. (Prentice-Hall, Englewood Cliffs: New Jersey and Hemel Hempstead, UK, 1979.) *Biochemistry.* By F.B. Armstrong and T.P. Bennett. Pp.491. (Oxford University Press: Oxford, 1979.) £14.

THE stated objective of Davies and Littlewood is to show "that biochemistry is fun" in a book aimed at students not specialising in biochemistry. Whether it is fun or not, the result is a fine book that would make an adequate textbook for any student whose course does not demand the mastery of one of the larger standard textbooks or who wants firstly to read a simpler account of the general features of the subject.

The book, consisting of 16 chapters, has 346 pages of quite small print. The style is simple and attractive and the figures are clear. The scope of the book is quite wide, opening with a chapter on cell structure and organisation, organic biomolecules and the elemental composition of cells. Three chapters are then devoted to protein structure and properties, determination of protein structure (protein separation, sequence determination, X-ray crystallography) and a simple description of enzyme function. The chapter on enzymes does not make recourse to a single equation but manages to describe the salient features of enzyme action together with sections dealing with the use of enzymes in medical diagnosis, industrial uses of enzymes, and enzyme inhibitors. The latter section is interesting since the actions of a number of poisons are discussed together with the actions of a number of antibiotics as enzyme inhibitors.

The general features of intermediary metabolism are covered in approximately 120 pages including, in the section on lipids, discussion of membranes, lipoproteins and the involvement of lipids in heart disease. Two chapters are devoted to nucleic acid structure, transcription and translation including a small section on the use of recombinant DNA in 'genetic engineering'. Human genetic diseases are also briefly considered in this part of the book. Regulation and control are specifically dealt with in a separate chapter which introduces the concept of the operon using the control of the expression of the *lac* operon as an example, briefly considers regulation of enzyme activity and contains a section on hormones. This latter section, which considers plant hormones and pheromones as well as mammalian hormones, is probably too brief and superficial to deal adequately with the topic but does contain some useful summary tables of hormone structure, source, effect and symptoms of excess or deficiency. The final two chapters in the book are unusual for an elementary biochemistry text. One deals with viruses (structure, life cycle and possible involvement in cancer), the other with antimicrobial agents. There is also an appendix setting out some important aspects of organic chemistry that are relevant to biochemical reactions.

In conclusion, the authors have done well in producing a very sound book which may well appeal to a wide readership.

Armstrong and Bennett's is a general textbook on the subject containing more material than the book by Davies and Littlewood but considerably less than the established textbooks by Lehninger, McGilvery, Stryer, and so on. 467 pages are contained in 24 chapters but the quantity of text on each page being quite small together with a large number of figures means that the content is less than at first sight. The contents include the history of biochemistry, protein structure, simple enzyme kinetics, carbohydrate, lipid and nucleic acid structure (one chapter on each), coenzyme function, six chapters on metabolism (including one on the basics of human nutrition), a chapter on hormones, and three chapters covering molecular genetics and protein synthesis.

The text is clearly and concisely presented and the figures are good. However, I found the index rather incomplete and there are some serious omissions in the section on intermediary metabolism. Certain aspects of lipid metabolism in particular are grossly neglected. I could find no sections dealing with fatty acid and sterol biosynthesis or with ketone body metabolism. Glycogen metabolism is barely mentioned except when its regulation is used as an example of hormonal control. Gluconeogenesis also received scant attention with no consideration of its physiological role.

Priced at £14, I do not feel that this book will be competitive on the student market. □

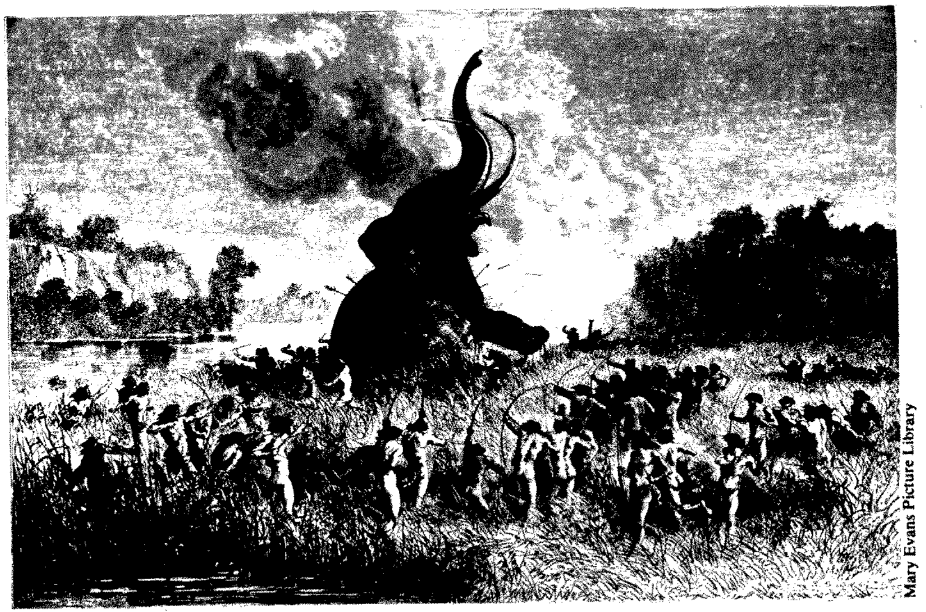
David Saggerson is Lecturer in Biochemistry at University College London, UK.

Food provision and energy resources

K.L. Blaxter

Food Energy and Society. By D. and M. Pimentel. Pp.165. (Edward Arnold: London, 1979.) Paperback £3.95.

THE dependence of food provision for man on sources of energy other than contemporary sunshine has been dealt with in a spate of books and articles published in recent years. The Pimentels' book, through numerous examples in the text and in tabular form, provides a wider basis of illustration of this dependence than most and in so doing a wider perspective. It is designed for undergraduate teaching and will be useful. Indeed, as much of the information quoted is given in original units, it will provide undergraduates with useful exercises in conversion of units to a common basis.



Early man solving the problems of food provision

Mary Evans Picture Library

The presentation commences with primitive hunting systems of providing food, progresses through simple types of agricultural systems to deal finally with modern ways in which food eventually reaches the dining table and the support energy costs of the many separate processes involved. Such a descriptive account should lead to ideas about strategies to deal with impending future problems. As have others, the Pimentels point to the necessity of stabilising population to stabilise food demand and to the desirability of replacing animal products by plant products in the human diet so to avoid the trophic axe associated with secondary production systems. These vegetable food products can certainly not be American canned corn for they show that to produce a can containing 455 g corn with an energy value for man of 1.6 MJ entails the expenditure of 12.6 MJ support energy as fossil fuel to process, package, transport, distribute, store and cook the corn. No doubt they had less sophisticated systems for the distribution of primary agricultural products in mind. It is a great pity that a book designed for undergraduates terminates on the pessimistic theme: "Although man's scientific expertise will help alleviate some of the world shortages, science cannot solve the problems the world faces today". It would have been better, having pointed out the problem, to have emphasised the challenge that that problem poses. □

Sir Kenneth Blaxter is Director of the Rowett Research Institute, Aberdeen, UK.

Marine biology

A.B. Bowers

Marine Biology. By J. Reseck. Pp.257. (Prentice-Hall International: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1979.) £10.75.

JOHN Reseck's book reflects his ideas on how marine biology should be taught to senior classes in schools and introductory classes in universities. The first part deals with physical and biological processes important to the understanding of life in the sea, the second with environments and ecological niches, and the third with taxonomy and characteristics of marine animals. He places equal importance on interesting and instructing students. The style is direct with short sentences and clear explanations, though word usage and sentence construction will often irritate grammatical purists. Each chapter begins with definitions of technical terms which might be unfamiliar to the reader, and ends with review questions to check his understanding of the material presented. The book is generously illustrated, with more black-and-white photographs than line diagrams. There is a selected reading list at the end and a good index.

The scope of the book is impressive: topics range from primary production to man's use and misuse of the sea, from polar seas to tropical mangrove swamps, and from protozoa to sea-birds and mammals. Most attention is given to North American environments and fauna; the algae deserve fuller treatment. Unfortunately there are many shortcomings in presentation of the material that could easily have been corrected before publication. Some figures are misplaced in relation to the text. The labelling of several important diagrams is inadequate or inaccurate. Many of the photographs are inappropriate; some of them would puzzle a specialist because the unusual viewpoint, poor contrast and lack of scale make them difficult to interpret. There are careless errors in the text.

The author states that the book is designed for the student to buy and read through, to be complemented by reference books from the library. I would certainly encourage teachers to buy it, but suggest that they make their own decision about recommending it to students in its present form. A carefully revised edition would be very useful. □

A.B. Bowers is a Senior Lecturer in Marine Biology at the University of Liverpool, UK.

Oceans of literature

R.I. Tait

Oceanography. By J.J. Bhatt. Pp.322. (Van Nostrand, Reinhold: Wokingham, UK, 1979.) £12.10. *Ocean Science*. By K.S. Stowe. Pp.610. (Wiley: Chichester, UK, and New York, 1979.) £9.50. *Exploration of the Oceans*. By J.G. Weihaupt. Pp.589. (Collier-Macmillan: London, 1979.) £12.75. *Descriptive Physical Oceanography*. By G.L. Pickard. Third edition. Pp.233. (Pergamon: Oxford and New York, 1979.) Hardback $25, £12.50; paperback $9.95, £5. *Introduction to Physical Oceanography*. By J.A. Knauss. Pp.338. (Prentice-Hall: Englewood Cliffs, New Jersey and Hemel Hempstead, UK, 1979.) £19.45. *Oceanography: Units 1-16*. The Open University Course Team. (Open University Press: Milton Keynes, 1979.) From £3.25 to £7.50 for each of 7 volumes.

To those involved in the teaching of oceanography at University, the choice of suitable text books poses many problems. For introductory courses the range of books available can be divided into two categories: (1) those which attempt to cover all aspects of oceanography; and (2) those which specialise in only one of the related disciplines. The latter group is primarily intended for students wishing to study a particular branch of oceanography or perhaps those who, while specialising in one oceanographic discipline, require a background in another. As courses at University level are usually devoted to one or two disciplines only, category 2 texts are the prime requirement. Books in group 1, on the other hand, normally fall under the heading of "background reading" only. There is an abundance of such books on the market. They are usually lavishly produced, with photographs and diagrams of the highest quality; a valuable source of visual aids to the teacher but, because of their somewhat superficial treatment of any particular topic, of limited use to the serious student. They do, however, play an important role in popularising the subject and as such they constitute a valuable part of the oceanographic literature.

The group of books reviewed here is divided between these two categories. *Oceanography* by J.J. Bhatt, *Ocean Science* by K.S. Stowe and *Exploration of the Oceans* by J.G. Weihaupt are all good examples of category 1. *Descriptive Physical Oceanography* by G.L. Pickard and *Introduction to Physical Oceanography* by Knauss fall into category 2. The last volume to be discussed, the Open University course *Oceanography, Units 1-16*, although belonging to the first grouping, has a different format: it is basically a students' work book and as such is unique.

The three books in category 1, Bhatt, Stowe and Weihaupt, all encompass the broad spectrum of the subject including the history of oceanography, the geophysical origins of the Earth and ocean, the physics, chemistry, geology and biology of the sea as well as related topics such as ocean management. All are up to date in their approach to the subject. Of the three, *Oceanography* by Bhatt is perhaps the most attractively produced. Basic definitions are given in a marginal glossary and each chapter concludes with a summary and suggestions for further reading. Effective use is made throughout of photographs to illustrate modern instrumentation.

One consequence of reviewing several books together is that their relative weaknesses become accentuated. Bhatt suffers in this respect. It is about half the size of the books by Stowe and Weihaupt and as it covers much the same field, depth of treatment must suffer accordingly. There is always a danger that too superficial a discussion of any topic may be misleading. To give one example, a student reading the chapter on ocean circulation in Bhatt will not gain an insight into the true nature of the Coriolis force; nor will he understand geostrophic currents, particularly as the illustrative figure is incorrect. And there are many other examples of sketchy treatment. However, in spite of this fundamental weakness there is much good material in Bhatt. Although not of a sufficiently high standard for a University course it will undoubtedly find a place in the school library.

The other two volumes by Stowe and Weihaupt are very similar in content and presentation. Both are eminently suitable for a general course in oceanography or as background reading for students at a higher level. In Weihaupt each chapter is followed by suggestions for further reading; Stowe gives only a summary and no references whatever! However, he does back each chapter with a list of study questions (over 100 in some cases) which is an excellent idea.

Following an introduction to the history of the subject, both texts give a good account of the geological-geophysical aspects of oceanography. Stowe is particularly good on geophysics: he treats the subject at a somewhat higher level than Weihaupt who on the other hand has added an interesting chapter on the changing level of the sea. Climatic aspects of the oceans are adequately covered in both books, but neither author gives sufficient attention to the heat budget of the ocean. Weihaupt is weaker on the dynamics of ocean currents. The T-S diagram receives insufficient treatment in both texts but waves and tides are adequately covered for the intended audience.

Both books have extensive and informative sections on marine biology. As a non-expert in this subject I found Weihaupt here perhaps easier to read. Each volume concludes with a stimulating chapter on ocean resources and man's impact on the ocean environment. There is little to choose between these two books. If I had to select one it would probably be Stowe, if only because he achieves overall a slightly higher scientific level than Weihaupt. However, both texts are very good. I would rate them as amongst the best books on general oceanography to appear since *The Oceans: Their Physics, Chemistry, and General Biology.* (Sverdrup *et al.*, Prentice-Hall: Englewood Cliffs, New Jersey, 1942).

Descriptive Physical Oceanography by G.L. Pickard is a well known oceanographic textbook which is now in its third edition. As a specialised text it goes more deeply into a physical description of the world's oceans than any of the books discussed above. Where appropriate, some minor changes have been made between the second and third editions to bring the subject matter up to date, but essentially text and diagrams remain unchanged. An important innovation, however, has been the conversion to SI units and the addition of a useful appendix on the units used in physical oceanography.

There is almost no mathematics in Pickard. The author's aim was to cover all aspects of physical oceanography which could be described in non-mathematical terms, with the intention that a companion volume would follow covering the dynamical side of the subject. This volume has at last appeared: *Introductory Dynamic Oceanography* (S. Pond and G.G. Pickard, Pergamon: Oxford and New York, 1978). Together with Pickard's descriptive oceanography it fills a long felt need for a basic text at undergraduate level, or for graduate students embarking on a career in physical oceanography. Whether description and theory should be divorced as completely as they are in Pickard, and Pond and Pickard, is a debatable point. My personal view is that it is a mistake. Integration of description with analysis can often be helpful in the understanding of the latter. (See Knauss below.)

In comparison with all the other books discussed here, the format of Pickard appears very drab indeed. Apart from the cheap production (intentional, but somewhat improved in the third edition) the most obvious shortcoming is the paucity of illustrations. The reader is often faced with unrelieved pages of text, whereas a few simple diagrams would have provided clearer insight with less tedium. I have little doubt that many students find it a rather dull book. However, in spite of this, I shall continue to recommend it to my students: the subject matter is good and presented at the right level for the descriptive part of an undergraduate course in physical oceanography.

In his *Introduction to Physical Oceanography* J.A. Knauss provides a text intended for "beginning graduate students and upper division undergraduates". As such it is set at a relatively high level of mathematical rigour compared with the other books. Use is made of vector notation and Cartesian coordinates throughout; some specialised derivations relating to the equations of motion and the wave equations are relegated to an Appendix which seems a good idea as it leaves the text free of excess mathematics. Another good point is the clear listing on the inside of both front and back covers of all symbols used. The Appendix also contains four pages of useful tables relating to the physical properties of sea water.

The book is very well written and the material is logically presented. The numerous illustrations of excellent quality are used skilfully in conjunction with a clear prose style, making even the more difficult concepts readily understandable. A reasonable balance is achieved between description and analysis and one of the features of the book is the use, particularly in the dynamical sections, of examples from the real ocean to illustrate theoretical points made. It is perhaps inevitable that not all sections of the book should achieve the same standard. Chapters which stand out as attaining a high level are those on the physical properties of sea water (with the appended tables), the heat budget of the ocean and the four chapters on ocean dynamics, although here some readers may be disturbed by the rather unorthodox development of the balance of forces "without Coriolis" followed by the balance "with Coriolis" instead of the usual treatment of forces "without friction" and "with friction". I cannot avoid mention of one unfortunate error: in Fig. 7.1 the direction of the geostrophic flow in three of the cells shown is reversed!

In other sections the topics are not treated to the same depth and the book as a whole cannot therefore be considered as giving a balanced view of the subject. Notable weaknesses are an inadequate treatment of boundary effects and insufficient development of the description of oceanic water masses and T-S analysis. The chapter on tides is also disappointing. However, a comprehensive text to the standard of the best chapters would require a much larger book. As it stands it is overall a good text which goes a long way towards filling a persistent gap in the literature.

The Open University interdisciplinary course (S.334) in *Oceanography* is probably one of the best publications available at present, particularly in view of its impressive attempt to teach, rather than merely describe, the basic concepts of the subject. It comprises seven sections totalling 780 pages that deal with the physical, chemical, biological and geological aspects of the oceanic environment, as well as an appraisal of the complex political and economic problems which have arisen due to the exploitation of marine resources. The presentation of all the material follows a format of discussion, questions, answers, comments, self-assessment and revision. It incorporates clear diagrams which have been either reproduced from well documented sources, or specifically constructed to illustrate the points under discussion. Each section concludes with a comprehensive list of references and suggestions for further optional reading.

To assimilate the contents of this course without too much difficulty a student would require a basic knowledge of at least two of the major sciences, and in fact would have a considerable advantage if one of these was in the field of geology since there is a distinct imbalance in favour of this subject. However, every effort is made to accommodate the less experienced by punctuating the text with a liberal number of self-assessment exercises. Wherever possible the reader is encouraged to recall and compare different facets, which are in fact interrelated, of an oceanic phenomenon, although previously these might have been treated solely as a particular physical, chemical, or biological event. Indeed, the student should be prepared, for example, to consider such diverse oceanic processes as those that control: the broad eastern boundary currents with their associated regions of coastal upwelling; the balance between oxygen concentration and biological consumption; the rates of production and growth of commercial fish stocks; the sediment accumulation in the deep ocean; and the present legal arguments regarding international straits, artificial installations and marine pollution. Whatever the topic, the information provided is invariably sufficient to enable the reader to gain an insight into the subject which is seldom achieved in the more superficial presentation of many oceanographic text books. The OU team have produced a most commendable script. □

R.I. Tait is Senior Lecturer in Oceanography at the University of Liverpool, UK.

Isotope geology

Grenville Turner

Lectures in Isotope Geology. Edited by E. Jager and J.C. Hunziker. Pp.329. (Springer: Berlin, Heidelberg and New York, 1979.) DM49.00, US$27.00

THIS book on isotope geology is the outcome of a two-week course of lectures to graduate and postgraduate earth scientists delivered by an international group of scientists at the University of Berne in 1977. It contains 24 chapters and covers topics related to the standard isotope dating methods (Rb-Sr, K-Ar, ^{40}Ar-^{39}Ar, U-Th-Pb and fission tracks) as well as the principles and applications of stable isotope geochemistry. According to the preface and flyleaf it is intended to be an introduction to isotope geology aimed at geologists rather than isotope specialists. My own feeling is that it is not a book to read without some prior knowledge. As a textbook for a course on isotope geology it does not compare at all well with the excellent book by Faure, *Principles of Isotope Geology* (reviewed in *Nature* **272**, 103 (1978)).

In fact, I was rather disappointed by this book. Before reading it I made a mental list of some of the topics in isotope geology which I regard as currently the most exciting. My list included the chronology and early evolution of the Earth's crust, the history of crust and upper mantle evolution based on modern studies of Nd-Sm, Rb-Sr and Pb isotopes, isotopic anomalies in the early Solar System and the chronology of the early Solar System. Apart from a short chapter on Archaean geochronology, an even shorter one on mantle geochemistry, and a discussion of crust and mantle interactions in the chapter on lead geochemistry, these exciting fields are virtually ignored. The contributions to the book are also rather patchy in the sense that 6 of the 24 chapters were of 5 pages or less (the shortest on the now defunct total lead method was 2 pages!). Two authors were particularly guilty in this respect. I feel that the editors should either have rejected some of the contributions or demanded more substantial work. I realise that to construct a coherent whole from a list of 17 contributors is not an easy task. Nevertheless, it is an essential one for a book intended to be used as 'an introductory course'.

This review has emphasised the bad points of the book; like the well known egg it has some parts worthy of consumption and several authors have given worthwhile summaries of particular fields. As a rough but not entirely correct guide, the usefulness of the chapters correlates with their length. Geologists and geochemists entering or already occupying the isotope field will therefore find parts of the book useful. However, considering its high cost I would encourage them to consult a library copy before buying. □

Grenville Turner is Reader in Physics at the University of Sheffield, UK.

Cloud physics

J. Latham

A Short Course in Cloud Physics. Second edition. By R.R. Rogers. Pp.248. (Pergamon: Oxford, 1979.) Hardback £14.50; flexi £7.25.

THIS short book is written for postgraduate students entering research in cloud physics. The structure of the book is conventional and logical. The initial chapters are concerned with meteorological thermodynamics. The appropriate thermodynamic laws and equations are first developed for dry air and are then extended to take account of the presence of water vapour. This leads to a discussion of static stability and air parcel buoyancy, which evolves naturally into an account of current understanding of mixing of air masses and theories of convection.

Three chapters are devoted to non-freezing clouds. The elements of homogeneous nucleation of water droplets are presented and then extended to take account of the role of condensation nuclei in the atmosphere. The subsequent growth of stable droplets by vapour diffusion is discussed and attention drawn to recent developments in the molecular kinetics of this phenomenon. The formation of raindrops by the stochastic process of collision and coalescence of cloud droplets is considered. The parallel processes — heterogeneous nucleation, vapour growth and accretion — occurring in clouds containing the ice phase are then discussed. An account is presented of the relative growth rates of precipitation by the coalescence and ice crystal processes.

A chapter is devoted to various properties of precipitation particles. The size distribution of the hydrometeors occurring in natural rainfall and snowfall are discussed. The formation of satellite drops by the processes of aerodynamic breakup of raindrops or the collision of drop pairs is considered, and it is shown how the latter process produces spectra similar to those observed. The aggregation and breakup of snowflakes, and the precipitation rates encountered in various meteorological circumstances are then outlined.

A useful account is presented of the role of radar in meteorology and cloud physics research. A discussion of the basic principles of radar leads to development of the relevant equations linking the received power to the properties of the precipitation scattering the radiation. This chapter concludes with a description of types of radar display and special techniques employed in cloud physics, such as Doppler radar.

Two chapters entitled 'Precipitation

Processes' and 'Severe Storms and Hail' are devoted to more macroscopic aspects of cloud physics. Various precipitation systems are described, attention being given to the airflow which governs the release and distribution of rainfall. A brief discussion is presented of the efficiency with which cloud water is transformed into precipitation. A detailed account follows of the dynamics and life cycle of severe storms. This leads naturally to a description of the formation of hail and the energy balance equations which govern the growth of hail pellets.

The final two chapters contain skeletal descriptions of current works in the important areas of weather modification and numerical modelling of cloud processes.

The problems for students presented at the end of each chapter are imaginatively devised and well balanced. It is helpful that in this second edition answers are given to odd-numbered questions. The book is modestly priced and serves as an excellent introduction to cloud physics. □

J. Latham is Head of the Physics Department of UMIST, Manchester, UK, and President of the International Commission on Atmospheric Electricity.

Inorganic analysis

F.J.C. Rossotti

Vogel's Textbook of Macro and Semimicro Qualitative Inorganic Analysis. By G. Svehla. (Longman: Harlow, UK, 1979.) £14.95. *Practical Inorganic Chemistry.* By G. Pass and H. Sutcliffe. Pp.242. (Chapman and Hall: London, 1979.) Paperback £3.95.

FOUR editions of Vogel's textbook appeared between 1937 and 1954, the heyday of qualitative inorganic analysis in British schools and universities. Twenty-five years later, Svehla has produced a fifth edition, in an age when less time is devoted to practical chemistry in British curricula and the popularity of systematic qualitative analysis has waned. One of the benefits of a sound grounding in qualitative analysis is that students become familiar with a great deal of phenomenological inorganic chemistry, for example, colours, solubilities and reactions in solution. A backwards swing of the pendulum would now be timely.

The systematic qualitative analysis of some 44 cations and 47 anions is described. Length and contents of the new edition are much the same as before. Two short chapters on interfering anions and on paper chromatography in the previous edition have been assimilated into other chapters and there is some shuffling of order. However, updating seems to have been modest. Thus the section on solvent extraction remains weak and largely irrelevant in its detailed content. It might, for example, have been helpful to students to inform them that systems of qualitative analysis, which rely entirely on solvent extraction, now exist. In this connection, citation of key references (as in the Vogel *Quantitative Inorganic* text) would have been helpful in much of the book.

The most disappointing feature of the new edition is the still antiquated treatment of reactions in solution. Thus ammonia and copper (II) ions do not yield a unique tetra-ammine (Dawson, 1900) but rather five mononuclear ammines in stepwise equilibrium (Bjerrum, 1931). The blood-red colour produced on mixing iron (III) and thiocyanate ions has been variously attributed to $Fe(NCS)_6^{3-}$ (third edition), $Fe(NCS)^{2+}$ (fourth edition) and now to $Fe(NCS)_3$ (fifth edition). In fact, six thiocyanato complexes coexist in stepwise equilibrium and in relative amounts which depend upon the free thiocyanate ion concentration. Discharge of the red colour by fluoride ions is not due to the formation of a unique complex FeF_6^{3-}, but rather to an array of mononuclear fluoro complexes. Moreover, it is doubtful whether the hexafluoro species exists at all in aqueous solution. These typical outdated misconceptions about the nature of interactions between inorganic species in aqueous solution seriously mar an otherwise useful bench-book.

By contrast, Pass and Sutcliffe's text deals with the practical inorganic chemistry that has so largely replaced qualitative analysis in British university curricula. The current volume is a paperback reprint of the second edition of 1974. It contains a wide variety of preparative exercises, together with some reactions of transition metal ions and applications of some instrumental techniques. The interweaving of a certain amount of theory with the experimental instructions, together with the modest length and price of the book, must make it attractive for many courses.

Arbitrary choices of topic are inevitable if space is at a premium, but the omission of solvent extraction from a chapter on separation techniques, which contains ion-exchange, seems somewhat surprising. Again, it is regrettable that instruction on Job's method of continuous variations persists in the 2nd edition. This archaic method is of very limited applicability, again by virtue of stepwise complex formation. □

F.J.C. Rossotti is a Lecturer in Inorganic Chemistry at the University of Oxford, UK.

Plasma physics

I.H. Hutchinson

Physics of High Temperature Plasmas. Second edition. By G. Schmidt. Pp.408. (Academic: New York, 1979.) $29.50.

THE dilemma of most didactic texts, particularly in relatively rapidly developing subjects, lies between a presentation of the historical development of the discipline and an essentially axiomatic exposition along what are seen as the most logical lines. Few general plasma physics texts follow as completely the latter, non-historical approach as the second edition of *Physics of High Temperature Plasmas* by George Schmidt. This bias is emphasised by the deletion of the first edition's quite detailed bibliographies accompanying each chapter, in favour of a very brief list of books at the end. However, perhaps because of this approach, the new edition gives very little evidence of ageing.

The coverage is very wide, including treatments of particle motion guiding-centre approximations, the derivation of the CGL approximation, two chapters on magnetohydrodynamics, Vlasov wave and instability analyses, bounded plasma effects and touching on relaxation and collisions. The bulk of the material new to this edition is contained in a chapter on non-linear waves, providing a good introduction to topics not often covered by general texts.

The book is fully committed to being self-contained in its development. In this it succeeds rather well, hardly every specifically appealing to the reader's credence ("it can be shown"). However, there are definitely occasions when rigour is sacrificed in claiming the self-evidence of statements which, on reflection, are plausible though not obvious. Such points are inevitable and not necessarily detrimental. Indeed, greater attention to minutiae might have made the exposition too formal. As it is, while the many examples that are worked out in depth are mostly of direct experimental relevance, the uninitiated are left wondering just how the results really work out with a laboratory plasma. The feeling is enhanced by the fact that what few illustrations there are of the results in action are mostly of computational simulations.

A remarkable success of this book is that it covers such a broad range of topics with significant depth in just four hundred pages. This is achieved, on the one hand, by a very happy choice of examples and lines of development of the subject. On the other hand, though, the prose is compact, sometimes terse, and the mathematical development inexorable; so that all but the most mathematically accomplished student must find it heavy going at times. Nevertheless, there are many valuable physical insights to be gained and we rarely lose sight of the physics behind the equations.

There are an annoying number of errors and misprints for a second edition. "Stellerator" is a typographical error since it appears elsewhere correctly, but "Tokomak" seems deliberate and is neither a consistent transliteration of the Russian nor in conformity with general usage.

There is not really a wide choice of general graduate level plasma physics texts. Teachers will welcome this quite up to date edition and researchers and students continue to appreciate its insights and breadth. □

I.H. Hutchinson is a Research Plasma Physicist at the UKAEA, Culham Laboratory, Abingdon, UK.

Basic organic chemistry

John Mann

Fundamentals of Organic Chemistry. By A.N. Nesmeyanov and N.A. Nesmeyanov. Pp.484. (MIR Publishers/ Central Books: London, 1978.) £4.50 each, £16 for set of 4 volumes. *Organic Chemistry.* By D.J. Pasto and C.R. Johnson. Pp.542. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) Paperback £10.35. *Qualitative Organic Analysis.* By W. Kemp. Pp.183. (McGraw-Hill: New York and London, 1979.) Paperback £4.85. *An Introduction to Organic Chemistry.* By J. Carnduff. Pp.194. (Wiley: Chichester, UK, and New York, 1979.) £8.50.

THERE are no weighty tomes or glossy American textbooks under review this year. Instead, a Russian attempt in four, slim volumes, to cover the fundamentals of organic chemistry; a laboratory handbook of organic chemistry; a book of qualitative organic analysis; and an advanced school text.

The four-volume Russian work, *Fundamentals of Organic Chemistry,* by A.N. Nesmeyanov and N.A. Nesmeyanov is (I quote from the introduction) "designed for systematic study of Organic Chemistry, and covers a considerably wider field than a normal university treatment". Certainly the coverage is broader than in the average UK or American texts, and is more akin to that of Finar (*Organic Chemistry,* two volumes, Longmans: Harlow, UK, 1951) in its first edition. This resemblance extends to the old-fashioned representations of structures and stereochemistries, excusable in the ancient version of Finar, but not in a modern textbook.

Volume one commences with a full mathematical treatment of the theory of chemical reactions, and later does the same for electronic structure. Standard (in some cases antiquated) methods of preparation of saturated aliphatic compounds are then given, together with their main reactions, with not a curly arrow in sight. There are some good tables of physical constants, a rare sight in modern textbooks of organic chemistry; and the volume concludes with a discussion of the classical chemistry of unsaturated aliphatic compounds.

This leads, in volume two, on to optical isomerism, and some very poor representations of chiral molecules. It is here that the comparison with modern western textbooks is so stark: one only has to think of the splendid illustrations in Cram and Cram (*Essence of Organic Chemistry;* for review see *Nature,* **278,** 82; 1979), for example, to appreciate this fact. The reader is then led through sections on sugars (flat structures only), amino acids and polypeptides (no stereochemical structures at all), and alicyclic compounds. To be fair, this latter subject is dealt with in far greater detail than in any comparable text. The volume concludes with a chapter on physical methods of structure elucidation, comprising sections on mass spectrometry (here the reader is hurled straight into an exposition of Russian peptide sequencing using MS), nuclear magnetic resonance (again weak on fundamentals), and brief sections on infrared spectroscopy, and polarimetry. The comparisons with western textbooks are again rather unflattering.

Volume three includes aromatic and heterocyclic chemistry, and coverage of both topics is very extensive (295 and 121 pages, respectively). Some Russian specialities are stressed: many pages on the stereoisomerism of substituted biphenyls, and 44 pages on condensed aromatic systems.

In the final volume there is a comprehensive, if somewhat antiquated, treatment of organometallic chemistry, with a particularly extensive section on metal carbonyls (usually confined to inorganic texts). There is little mention of synthetic utility: hydroboration merits one page, whereas the Wittig reaction gets one mention in volume one, and half a page in this volume. Similarly, the section on carbanion chemistry contains no mention of current synthetic methodology. Terpenoids and alkaloids then receive a classical treatment, and the structures are particularly archaic here; but there is more steroid and alkaloid chemistry than in most student texts. However, the relevance of these secondary metabolites to pharmacology and ecological chemistry is ignored completely. The final chapters include discussions of nucleic acids, enzymes, and cofactors; and this is quite well done, apart from an appalling photograph of the myoglobin molecule (one of the few photographs in the book).

At its best this work is comprehensive though rather antiquated; but the overall impression is one of dullness — it lacks the vital spark and visual appeal of most western textbooks.

D.J. Pasto and C.R. Johnson have, in their new book *Organic Chemistry,* produced an excellent laboratory handbook for undergraduate chemists. The book is in three parts: basic laboratory techniques, spectroscopic methods, and qualitative analysis. The introductory chapter in part one starts from first principles, and leads the novice experimentalist through sections on laboratory safety, elementary first-aid, general equipment and its uses, a solvent list, and yield calculation. This is followed by a chapter on techniques of separation and purification, from recrystallisation, sublimation, and distillation, through to a good section on chromatography which includes HPLC. Part one concludes with a chapter on physical characterisation which is concise but adequate. It is worth comparing this part of the book with the first part of Vogel (*Textbook of Practical Organic Chemistry*; also reviewed last year: **278,** 82). This far weightier tome is much more comprehensive, but far less readable, whereas Pasto and Johnson have provided an introduction to the classical methods of organic chemistry in a form which students might even enjoy reading.

The second part commences with ultraviolet and visible spectra, then proceeds to a discussion of infrared spectroscopy, all of which is more than adequate for undergraduates involved in structure elucidation. The NMR section is very good, and includes ^{13}C-NMR, NOE, and shift reagents, as well as all of the fundamentals; and the subsequent section on mass spectrometry is excellent. There are seventeen useful NMR problems (^{1}H and ^{13}C), and ten mass spectra to interpret. Overall, the treatment is as good, if not better, than the standard texts of the day: Williams and Fleming (*Spectroscopic Methods in Organic Chemistry*; McGraw-Hill: New York and London, 1973), and Kemp (*Organic Spectroscopy*; Macmillan: London, 1975).

The final part deals with qualitative analysis, and once again the coverage is adequate, rather than extensive as in Vogel; but unlike the latter, IR and NMR data are interspersed to show how modern spectroscopic methods and wet analysis can go hand-in-hand. The book concludes with a section on use of the research literature. Throughout the book there are good reading references, and it deserves to become a sort of bible for undergraduates taking courses in experimental organic chemistry; but it probably will not achieve this exalted position, as at £10.35 for a paperback, there are cheaper alternatives.

One of these is by W. Kemp (*Qualitative Organic Analysis*), who has successfully integrated wet analysis and modern spectroscopic techniques, but the coverage is not quite so comprehensive as that of Pasto and Johnson. The tables of derivatives are, however, marginally more extensive. The section on spectroscopic methods is much poorer, and includes only the bare essentials of each technique. The aim of the book is nonetheless realised, in that it shows qualitative analysis as a vehicle for learning functional group chemistry. On balance, a chemistry undergraduate would probably do better to buy Pasto and Johnson, whereas ancillary students should find Kemp's book adequate for their needs.

The fourth book, *An Introduction to Organic Chemistry* by J. Carnduff, seeks to present organic chemistry for advanced level school students, from an electronic point of view. Thus the standard reactions of organic compounds are discussed with reference to the fate of the electrons involved. This is a refreshing departure from the norm, as most advanced level school texts seem afraid to include mechanisms and curly arrows. There are seven chapters which cover the fundamentals: 'electron accounting', nomenclature, stereochemistry (including chirality — perhaps a bit ambitious at this level), electronegativity, delocalisation, and classification of reaction types. This is accomplished very effectively, and is followed by chapters on organic compounds, including a useful chapter on organometallic species, as well as the usual list from hydrocarbons to carbonyl compounds by way of alcohols and amines. The section on aromatic chemistry is particularly good. Problems appear at the end of each chapter, with answers at the end of the following one. The final chapter summarises what has been said about ionic reactions. This is a book full of good basic organic chemistry presented in a modern (mechanistic) fashion, and schools will probably buy a cheaper paperback version. My one reservation is the virtual absence of examples showing the industrial and biological relevance of organic chemistry. The Nuffield advanced level books go to the other extreme, with almost total exclusion of mechanism. Surely, a compromise can be reached — perhaps next year? □

John Mann is a Lecturer in Organic Chemistry at the University of Reading, UK.

Fluid mechanics

T.N. Stevenson

Fluid Mechanics. By H.C. Lowe. Pp.228. (Macmillan: London, 1979.) Paperback £3.95.

FIRST-YEAR engineering courses in fluid mechanics are chosen from a vast range of topics. The material found in these courses would fill many volumes, and consequently any one volume of this size is unlikely to be adequate for any one course.

Each chapter in the book includes a short description of the physics and a cursory look at the theory for a particular topic. This is followed by a number of examples and specimen solutions. Some of the examples rely on the theory in this or earlier chapters, but most of them require a much deeper understanding of the subject. Questions are taken from the examinations of the North Staffordshire Polytechnic and the Council of Engineering Institutions. The author knows the syllabus for these courses and has made the approximations expected in those examinations. For the benefit of other students the author could have indicated how more accurate solutions could be obtained to some of the problems.

The book is written in note form with some of the symbols incomplete due to poor type. At least two of the examples have omissions which make them dimensionally incorrect and the vector notation used is not always correct. Some partial derivatives have been typed as total derivatives. The sections on dimensional analysis, wakes, vortex shedding, and on turbulence are misleading. The explanation of Buckingham's π theorem is incomplete and the definition of laminar flow is wrong.

There are scores of books covering parts of first year undergraduate courses in fluid mechanics. Probably not one of these covers exactly the same topics as this text. There is an interesting set of examples in the book. However, in view of the omissions and errors in the explanation of the physics involved, I cannot recommend the book to students. □

T.N. Stevenson is Senior Lecturer in the Department of the Mechanics of Fluids, Honours School of Aeronautical Engineering, University of Manchester, UK.

Classical mechanics

J.W. Humberston

Intermediate Classical Mechanics. By J. Norwood, Jr. Pp.417. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) £14.30.

THE author's justification for writing another undergraduate text on classical mechanics is his belief that the subject should not only be concerned with particles and rigid bodies, but also with many body systems. He has therefore included chapters on elastic bodies, statistical mechanics and fluid mechanics in addition to the usual topics contained in books on classical mechanics. Some of the topics in these chapters are occasionally discussed in mechanics books at this level (for example, *Mechanics* by K.R. Symon), but others, such as plasma physics and hydromagnetic waves, are not. A chapter on relativity theory is also included which gives a rather superficial account of special relativity and only a very brief introduction to general relativity. Several short problems are set throughout the book, but no solutions are given.

The general level of presentation is somewhat higher than is usual for a first year course in mechanics in a British University, although anyone who has taken such a course should have little difficulty understanding most parts of the book. It is clearly written and very well produced and seems to contain very few misprints or errors. One rather unfortunate example of the latter, however, occurs in the discussion of Kepler's laws of planetary motion. The author states, and claims to prove, that the second law, the constancy of the areal velocity, is only true for an attractive inverse square law of force; it is, of course, true for any central force, being a direct consequence of the conservation of angular momentum. □

J.W. Humberston is a Lecturer in Physics in the Department of Physics and Astronomy at University College London, UK.

Statistics and experimental design in psychology

Donald Laming

The Numbers Game. By J.G. Snodgrass. Pp.467. (Oxford University Press: Oxford, 1979.) £5.95. *Experimenting in Psychology.* By R. Gottsdanker. Pp.352. (Prentice-Hall: Hemel Hempstead, UK, 1979.) £9.45.

IN teaching statistics and experimental design to psychology students there is always the problem of maintaining the students' interest. Here are two very different approaches to this problem.

The Numbers Game is a conventionally ordered introductory text. It begins with four chapters on descriptive statistics and then proceeds to an exposition of the standard normal-variate tests; non-parametric procedures are presented in the final three chapters. The writing of individual chapters reflects a great deal of thought and experience in teaching students who may have but little mathematical background. The presentation is conceptually oriented, with important mathematical arguments placed in supplements at the ends of chapters, and the concepts are illustrated with examples. In its concern for communicating with the student *The Numbers Game* is to be commended. But, because of the conventional ordering of the material, the student must work as far as chapter 10 before he is able to appreciate in its entirety even the simplest experiment. As Gottsdanker puts it in his preface to *Experimenting in Psychology:* " . . . to treat various "topics", such as *measures of behavior, subjects, experimental designs, and treatment of data* in separate chapters. When that is done, there can be no comprehensive knowledge about any kind of experiment until the last chapter is covered.".

Experimenting in Psychology employs a completely different approach. It is organised around a graded sequence of example experiments. These experiments are not used to illustrate previously stated principles (as in *The Numbers Game);* rather, they "carry" the exposition. Each experiment presents a practical problem which is worked through in the text, and the solution to this problem exemplifies the principle which the author wishes to present. In the early chapters the experiments are necessarily simple, mostly concerned with applied problems. Regrettably some of these are artificial, made-up examples, which are never as interesting as real ones. But in the later chapters dealing with the more complex problems of design, the experiments are all recently published examples drawn from a wide range of psychological subject matter. The reader thereby obtains some appreciation of the complexity of experiment needed in contemporary research and of why its design is important; in addition, as a by-product, the book encourages a catholic interest in scientific psychology.

Of these two books, I am confident that the second will hold the students' interest the more readily. From the outset it demonstrates the importance of experimental design in psychological research and provides the student with a motive for studying its technicalities. Dr Gottsdanker is to be commended for developing this new approach. But, at the same time, he has bent over backwards trying not to alienate those with a distaste for mathematics. The main text is entirely devoid of algebra and only a very little statistical theory is included, set in a series of statistical supplements placed at the end of each chapter. The author has, by intention, written a book on experimental design alone, virtually without statistics. But the criterion of good experimental design is that the data can readily be analysed with respect to the questions of interest; so that the possibilities for design are dominated by the analytical techniques. Dr Gottsdanker could have slipped in much more about data analysis than he has; it is a pity — it would have made the book more widely useful.

There remains the possibility that these two books, so very different in approach, might actually complement each other and be successfully used in tandem. They will provide an attractive combination for any psychologist who teaches chiefly the use of normal-variate procedures. ☐

Donald Laming is a Lecturer in Experimental Psychology at the University of Cambridge, UK.

Establishing psychology

O. L. Zangwill

Introduction to Psychology. By E. Hilgard, R. A. Atkinson and R. C. Atkinson. Seventh edition. Pp. 653. (Harcourt Brace Jovanovich: New York and London, 1979.) Hardback £10.35; paperback £7.75. *Basic Writings in the History of Psychology.* By R. I. Watson. Pp. 420. (Oxford University Press: New York and Oxford, 1979.) £5.95. *Psychology Survey, No. 2.* Edited by K. Connolly. Pp. 288. (George Allen and Unwin: London and Boston, 1979.) Hardback £7.50; paperback £3.95. *Brain Behaviour and Evolution.* Edited by D. A. Oakley and H.C. Plotkin. Pp.237. (Methuen: London, 1979.) Hardback £9; paperback £4.95.

THESE four books, two British and two American, are all concerned in one way or another with the teaching of psychology at University level. It is, however, virtually impossible to compare them directly on account of the very real differences that exist between the British and American systems of higher education. While the American texts are designed to meet the needs of College students taking an introductory course in psychology who may or may not wish to proceed further with the subject, the British texts are intended for second- or third-year students taking psychology as a main subject in an Honours Degree course in a British university. Although the teaching of psychology in Britain makes extensive use of American texts and journals, it cannot be said that either of the two American volumes under review is likely to prove obviously appropriate for use in this country. This is not, however, to say that they are both without educational value or do not merit sympathetic consideration.

The Hilgard, Atkinson and Atkinson text, originally published in 1953 and already fairly well known in Britain, now appears in its seventh edition. As in the earlier editions, the authors explain that they have tried to write an introductory text for students who in their experience are more likely to be interested in what they consider to be "relevant to their lives, futures and the problems confronting society" than in the preoccupations of professional psychologists. This fashionable demand for "relevance" they have attempted to meet "without sacrifice of scientific rigour or scholarship". In fact, the book consists very largely of the standard stuff of contemporary academic psychology enlivened by some reference to topics such as hypnosis, psychotherapy and the effects of psychotropic drugs, which students are presumably expected to deem "relevant". In spite of this somewhat patronising attitude to supposed student interests, it must be said that the authors have produced an attractive text that presents the subject matter of experimental psychology intelligently and well. It may be surmised that it owes much to Dr Hilgard's admirable good sense and solid judgement and to his fortunate choice of collaborators.

Dr Watson's compilation of *Basic Writings in the History of Psychology* at all events makes no compromise with "relevance", as it might be supposed that few American students of introductory psychology would dissent from the celebrated judgement of Henry Ford. The book consists of some fifty extracts from the writings of assorted philosophers and scientists, including Descartes, Locke and Helmholtz, who may be said to have laid the groundwork for the evolution of psychology as a science. It also contains extracts from the work of some thirty leading psychologists of the nineteenth and early twentieth centuries, mainly German and American although a few British. In his selection, Dr Watson seems to have been much influenced by E. G. Boring's views and follows closely the judgements expressed in his well known *History of Experimental Psychology*. As a result neither French nor Russian psychologists (with the exception of Pavlov, who would have hated to be called a psychologist) find inclusion.

While one may agree with Dr Watson that historical knowledge is of educational value, one may doubt whether the method he has chosen to represent it is a satisfactory one. Indeed it would seem to be modelled more upon *The Readers' Digest* than upon any serious contribution to historical scholarship. Nonetheless, there is just a hope that it might inspire an exceptional student to delve into some of the original sources. It is also exceptionally cheap by today's standards.

Let us now turn to the two British productions. *Psychology Survey, No. 2,* the second volume in a new series, follows closely the pattern established by Professor Brian Foss, who edited the first. It consists of fifteen chapters, each dealing with a single issue or area of inquiry in contemporary psychology. The (very varied) topics have been chosen by Professor Connolly on the grounds of their timeliness and promise on the one hand and the fact that their authors are personally engaged in research in the relevant area on the other. These chapters have no explicit connecting link and may be thought to illustrate well the late Professor Gilbert Ryle's view that psychology is less a science than a consortium of inquiries and techniques that neither has, nor needs, a logically trim statement of programme. Nonetheless, the editor clearly hopes that his patchwork quilt will provide both stimulation and variety. In this, he is unlikely to be disappointed.

The individual contributions to this book vary very considerably in their range of coverage. Some deal with large areas of the subject as, for example, human engineering or psychopharmacology; others with special issues or topics, such as the theory of colour vision or motor activity mutants of *Drosophila*. In (almost) all cases, however, the authors clearly know what they are talking about and express themselves lucidly and well. In particular, there is a merciful absence of jargon. Like its predecessor, this book may be expected to receive a warm welcome from teachers of psychology and their students.

Lastly we have *Brain, Behaviour and Evolution,* a collection of longish essays expressly compiled to provide students of psychology or the social sciences lacking a background in the natural sciences with some understanding of evolutionary biology. The chapters are of two types, the first four providing basic information about the anatomy of the nervous system, the evolution and genetics of behaviour, brain—behaviour relationships and neurochemistry in relation to evolution. The next five chapters are concerned with more specialised issues, including the evolution and functions of sleep, the relationship of brain size to intelligence (this is a particularly thoughtful and scholarly survey) and the ostensible functional asymmetry of the two cerebral hemispheres in man. To this last chapter Dr Roger Sperry appends a brief afterword on consciousness.

This book is on the whole well done and will undoubtedly be of help to the not inconsiderable number of students to whom it is directed. From an educational point of view, however, it must be insisted that such a book is no substitute for a basic grounding in the established biological sciences and one may well doubt the wisdom of allowing candidates to proceed to degrees in psychology or behavioural science without some previous grounding in the biological or pre-clinical sciences. If this should become an accepted convention, university departments of psychology would of course attract far fewer people than at present and staff and resources would correspondingly diminish. Nonetheless, the quality of the product might well be higher and the prospects for the subject brighter. Psychology would then have the chance to establish itself firmly in the consortium of biological sciences. ☐

O. L. Zangwill is Professor of Psychology at the University of Cambridge, UK.

For the newcomer to nuclear physics

Daphne F. Jackson

Theoretical Nuclear Physics. By J. M. Blatt and V. F. Weisskopf. Pp. 864. (Springer: New York, Berlin and Heidelberg, 1979.) DM64; $35.20.

THIS book was first published as a postgraduate textbook in 1952, twenty years after the experimental discovery of the neutron. Before its publication, newcomers to the study of nuclear physics would probably have used review articles published in the 1930s in *Reviews of Modern Physics* and *Handbuch der Physik* as their introduction to the subject, together with the more descriptive book by Bethe and Morrison.

Blatt and Weisskopf set out to give a complete presentation of the theoretical concepts and considerations which must form part of any theory of nuclear properties and of theoretical techniques which should be used in the analysis and correlation of experimental data. The latter aspect was of particular importance because nuclear physics at that time was in much the same position as atomic physics some thirty to forty years earlier, with a substantial accumulation of spectroscopic information and low energy scattering data but no means of deriving the behaviour of nuclear forces or the properties of nuclei from more fundamental sources.

The authors began at the beginning by discussing the constituents of nuclei, the stability of nuclei, and the significance of the behaviour of the binding energy per nucleon as a function of mass number. They made the assumption that non-relativistic quantum mechanics could be applied to nuclei and proceeded to discuss the excitation of nuclear states and the implications of quantum statistics for nuclear systems. These are all topics which have now reached well down into the undergraduate syllabus for physics students.

The major sections of the book which deal with two-body scattering at low energies and the basic character of the nuclear force and with the general theory of low energy nuclear scattering and reactions, were of great importance when first published and remain very valuable. It is remarkable that much of the notation and many of the illustrations have been carried on into later books by other authors. This confirms the success that Blatt and Weisskopf had in achieving their aim of making the subject understandable to readers equipped with a reasonable background in quantum mechanics. The section of the book dealing with the interaction of nuclei with electromagnetic radiation and the appendix on multipole radiation are outstanding.

The chapter on β-decay remains useful while that on α-decay is rather meagre. Fission is not covered in any detail because much of the relevant information was still classified at the time of writing. From the point of view of the modern reader, the least satisfactory sections of the book are those concerned with the interpretation of nuclear spectroscopic data in terms of nuclear models. When the book was started the shell model had only just emerged and it was nearly ten years later that Weisskopf exposed the important role of the exclusion principle in limiting two-body collisions of the bound nucleons and so making possible the long mean-free-paths required for the existence of independent particle motion. It then became possible to understand how the repeated influence of the residual interactions not included in the averaged potential of the shell model could lead to the formation of the compound nucleus. The addition of a short appendix covering this point would have helped new readers to avoid unnecessary confusion.

The book inevitably contains little discussion of reactions above 20 MeV and it is rather amusing to see energies between 10 and 50 MeV classified as "very high energies" and those above 50 MeV classified as "ultra high energies".

It is open to question whether the re-issue of a book which was written nearly thirty years ago, without any revision or even up-to-date commentary in footnotes and appendices, provides a real service to nuclear physicists. The book still provides a very good introduction to the theoretical techniques of low energy nuclear physics and could usefully serve as a background reader for a first major course on nuclear theory. The book would be of little further use to postgraduates working on nuclear structure theory or direct reactions but should remain of some value to experimentalists working on very low energy reactions and electromagnetic interactions. Indeed, the book might now serve as a foundation for studies in applied nuclear physics.

From a less utilitarian standpoint, there are strong reasons for congratulating the new publishers for keeping this major work in print. It is a classic example of how to write a textbook in a newly developing field. The style is lucid and the authors were capable of being both imaginatively descriptive and searchingly critical. The mathematical formalism has an elegant, uncluttered appearance. This book is attractive to an extent unsurpassed by later works of comparable scientific complexity. □

Daphne F. Jackson is Professor of Physics at the University of Surrey, UK.

Atomic, nuclear and elementary particle physics

P.G. Murphy

The Structure of Matter: An Introduction to Atomic, Nuclear and Particle Physics. By R.M. Turnbull. Pp.266. (Blackie: Glasgow and London, 1979.) Paperback £7.95.

THIS book contains a concise account of atomic, nuclear and elementary particle physics, for the early years of an undergraduate course in science. It is written at a fairly elementary level compared with most of the numerous texts of this kind. It gives more space than is usual to elementary particles, justified by the inclusion of very recent developments of fundamental significance. Most of the book contains traditional material treated in an orthodox manner. It should therefore reach a very high standard to justify its addition to our libraries, or the expenditure of a large sum for a slim volume. Let us therefore look at the treatment of one of the most important parts of the subject. On page 96 we are told that "Schrödinger postulated a wave equation . . .", followed in the next sentence by equation 8.5, which is a wave equation; nothing is said to prevent the innocent student from believing that it is the Schrödinger equation. Unfortunately it is not (except possibly the relativistic form for a massless particle). The true Schrödinger equation does appear, two pages later, as a result of some unexplained manipulations on a harmonic wave. Then we are told that it is "known as the time-dependent form since . . . V is in general a function of both position and time". This is wrong; the phrase refers to the wave function, not to the potential (which could well be zero). The next sentence says "Wave-particle duality is incorporated . . . since it . . . contains a potential energy term describing the interaction of a particle with its environment". This duality is inherent in the interpretation of the wave function and has nothing to do with the potential.

Tutors who have to eliminate these ideas will wish that this book had not been published. I have described only the worst faults I have found. In other parts we find an inadequate treatment of the uncertainty principle, a long section on Wilson-Sommerfeld orbits which is an unnecessary difficult subject, again with misleading and inadequate explanations (it leaves us thinking that Sommerfeld's relativistic term explains fine structure correctly), and other inaccuracies. This book cannot be recommended. □

P.G. Murphy is Professor of Physics at the University of Manchester, UK.

Developments in physics

Leonardo Castillejo

The Structure of Matter. By S. Gasiorowicz. Pp.525. (Addison-Wesley: London, 1979.) £16.50.

IN 1933 the publication of Max Born's classical book *Atomic Physics* set a standard for a comprehensive yet simple introduction to the new ideas and developments in physics. Since that time the subject has grown so large that most authors choose to restrict themselves to one field such as relativity or quantum mechanics, solid state or statistical physics, and to provide within the chosen topic more mathematical details and worked examples to enable the student, not only to grasp the new ideas, but also to calculate things for himself.

In this textbook on *The Structure of Matter* Dr Gasiorowicz has combined both these aims. He includes all the topics listed above, together with a wide range of simple applications and illustrations in related fields. The mathematical requirements are few; basic calculus and the use of matrices. The physical arguments are presented very clearly and followed up with explicit calculations, often on a simple model, so that all the details can be worked out. The crucial features which generalise to the real problem are then clearly emphasised.

The book is divided into six parts. The first covers relativity. This is an excellent and lucid exposition of the essential ideas and provides a working knowledge of Lorentz transformations, relativistic kinematics and even some transformations of electromagnetic fields. The second section on statistical physics is based on a mixture of statistical distribution and kinematic theory, derives the Boltzmann distribution, with some applications, and discusses black-body radiation. I was unhappy that the concept of temperature, which is so fundamental, was introduced rather superficially in terms of the ideal gas law.

There follow three sections on quantum mechanics; the first explaining the early models, then a self-contained and beautifully clear section formulating quantum mechanics up to the harmonic oscillator and finally a more advanced section including angular momentum, the hydrogen atom and spin. In the final part of the book the methods of quantum theory, statistical physics and relativity are used to describe and explain a broad spectrum of physical phenomena. The examples are well chosen to give an overview of modern physics as well as to provide opportunities to extend calculational techniques. For example, the discussion of electromagnetic transitions and selection rules also provides a derivation of time dependent perturbation theory, and the calculations of specific heats of phonons and electrons provide an introduction to Bose and Fermi-Dirac statistics. This last part introduces the reader to atomic and molecular physics, solid state, nuclear physics and elementary particles. The latter topic is treated in more detail than the others and ends with a discussion of quarks, colour and charm.

This book is exciting to read, yet designed for instruction and as a course text. There are many good examples at the end of each chapter. It is one of the best introductory textbooks to modern physics which I have seen and it deserves to be widely used. □

Leonardo Castillejo is Professor of Physics at University College London, UK.

Analytical chemistry

T.S. West

Analytical Chemistry. By D.J. Pietrzyk and C.W. Frank. Second edition. Pp.700. (Academic: New York and London, 1979.) $16.50.

A DETAILED examination of the text of this book shows that in many respects it is rather like the legendary 'curate's egg'. At first sight there are many excellent features of planning and intention such as material on sampling, choice of analytical method, literature on analytical chemistry, units, standards, preliminary operations, and so on; but, unfortunately, these are vitiated by lack of penetration into the subject or by a loose approach. So, what looks promising when one first looks at the list of contents, turns out to be little more than a run-of-the-mill treatment. In the sense that there are references to the edicts or regulations of bodies such as the FDA, EPA, APHA, ASTM, and so on, which is, of course, perfectly appropriate for the student mass market in the USA, the book is nevertheless a US national rather than an international publication.

It is considerably less welcome, however, to find the use of the 'Formality concept' predominating in the book and, even though lip service is paid to SI, it has to be said that 0.1F H_3PO_4 is more inconsistent and confusing than continued use of 'ml', 'litre', and so on, which are, at least, still in common use throughout the world. It is also noticeable that principal references are made almost exclusively to American papers and books and, indeed, that there are very few references to the original literature throughout the text. Students who use this text are consequently likely to receive little training or introduction to a concept which the authors apparently value highly. According to page 18: "The literature of analytical chemistry, as in any other scientific area, is the life blood. The books provide the newcomer with the required background while the journals provide a vehicle for critical discussion about the old and the new.". Yet on the very next page one of the leading international journals is incorrectly given.

The subject matter is treated fairly fundamentally and goes into the conventional techniques of gravimetry, titrimetry (given as "volumetry"), fairly well. The treatment of optical procedures such as flame spectrometry (still showing detailed diagrams of a total consumption burner), atomic absorption and fluorescence spectroscopy is so rudimentary that it is almost without value: similarly polarography. The separation section on ion exchange and chromatography is more satisfactory; but the solvent extraction chapter, which includes a solitary incomplete reference, is also lacking in penetration.

This is not a book that I would recommend with any degree of confidence.

T.S. West is Director of the Macaulay Institute for Soil Research, Craigiebuckler, UK.

Solid-state chemistry

J.N. Murrell

Structure and Bonding in Solid State Chemistry. By M.F.C. Ladd. Pp.326. (Wiley: New York and Chichester, UK, 1979.) £16.50.

THIS is a welcome book on solid-state chemistry, for I am sure that the subject gets short treatment in most chemistry courses. The difficulty faced by an author in this field is to decide what to include and what to omit. The subject cannot be taught without reference to gas phase molecular structure and to chemical bonding theory but these are topics adequately covered in other monographs. If this background material is omitted then I suppose that there is the danger that the book will either be rather thin or will have to include detail which is too specialised for an undergraduate text.

Dr Ladd has chosen to include a substantial amount of background material on basic quantum mechanics and the theory of the covalent bond (over 70 pages in all). He also includes 10 pages of appendices covering the thermodynamics of electrolyte solutions, required, as far as I can see, only for brief discussion of the solubility of ionic compounds. My own inclination would have been to direct the reader to other books for these topics because I think it diverts attention from the main direction of the text.

Chapter 1 is titled "Preamble" and gives an introduction to the structure of solids. In contrast to my view expressed above I found this section surprisingly thin on the experimental and theoretical aspects of diffraction. For example, I found no reference to neutron diffraction and its relevance to the position of hydrogen atoms in solids. The book would certainly not be adequate to cover the material necessary for undergraduate chemistry teaching of diffraction.

Chapter 2 covers ionic crystals comprehensively, reflecting one of Dr Ladd's research interests. There is a short section on colour centres. Chapter 3 deals with covalent compounds but only one page out of seventy deals with covalent solids directly (diamond, for example), the rest being used for the background material already referred to.

Chapter 4 is titled "Van der Waals' Compounds" and contains a brief description of intermolecular forces and their relevance to inert gas solids, organic molecular solids and donor-acceptor complexes. Chapter 5 covers metallic compounds and contains an elementary treatment of band theory; the free-electron model, Brillouin zones and Bloch theory. Each chapter presents a set of problems and solutions are given at the end of the book.

In summary, I feel that the balance of the book is not ideal but it should still be very useful reading for students of chemistry and chemical physics. □

J.N. Murrell is Professor and Dean of the School of Molecular Sciences, University of Sussex, Brighton, UK.

Astronomy textbooks

David W. Hughes

The Solar System. By J.A. Wood. Pp.196. (Prentice-Hall International: Hemel Hempstead, UK, 1979.) £5.05. *Galaxies: Structure and Evolution.* By R.J. Taylor. Pp.203. (Taylor and Francis Wykeham Publications: London and Basingstoke, 1979.) Paperback £4.50. *The Astrolabe.* By H.N. Saunders. Pp.12. (Science Studio: Oxford, 1979.) £4.75. *Projects and Demonstrations in Astronomy.* By D. Tattersfield. Pp.331. (Stanley Thornes: Cheltenham, UK, 1979.) *Astronomy: Activities and Experiments.* By L.J. Kelsey, D.B. Hoff and J.S. Neff. Pp.187. (Kendall/Hunt: Dubuque, Iowa, 1979.) *Activities in Astronomy.* By D.B. Hoff, L.J. Kelsey and J.S. Neff. Pp.142. (Kendall/Hunt: Dubuque, Iowa, 1979.) *Astronomy: From the Earth to the Universe.* By J.M. Pasachoff. Pp.200. (W.B. Saunders: Philadelphia, London, Toronto, 1979.) (Plus *Teachers Guide.*) *Exploring the Cosmos.* By L. Berman and J.C. Evans. Second edition. Pp.89. (Little, Brown: Boston and Toronto, 1979.) (Plus *Instructor's Manual.*) *The Macmillan Dictionary of Astronomy.* By V. Illingworth. Pp.378. (Macmillan: London and Basingstoke, 1979.) £8.95.

THIS year's crop of astronomy textbooks can be easily sorted into three groups which, for want of better, can be labelled advanced, experimental and simple.

Two books fall into the advanced section; both are on specific astronomical topics and both are aimed at the second- and third-year university undergraduate. Both are excellent in their own way and both are extremely good value for money. The first is *The Solar System* by John A. Wood, an ideal introduction for all astronomy, geophysics and physics students. Wood had an extremely difficult job in front of him — express in about 50,000 words and 125 figures what you think is of major importance in the Solar System. Now one easy way out is to isolate the planets from the Sun and the neighbouring stars in the Galaxy. Wood didn't do this, thank goodness, and this makes the book even more praiseworthy. He also decided to discuss, chapter by chapter, the unifying features of planets. So we don't get a chapter on each and an insulation of our knowledge of a single planet from the overall picture. Wood considers broad topics, the motions of planets and their interrelations, the surfaces and their evolution, planetary interiors and atmospheres, rocks from space (meteorites and lunar samples), the Sun, solar evolution and the origin of the elements and finally the origin of the Solar System. This book is going to be a firm favourite in universities for years to come. Maybe in the next edition Wood could help the lecturer out when it comes to tutorial and exam time by including a set of questions. Also the suggestions for further reading are a bit dated.

The second advanced book is *Galaxies: Structure and Evolution* by R.J. Tayler, a new title in the excellent *Wykeham Science Series.* Despite their intended aim — to bridge the gap between school and university studies — this book is again at mainstream second- and third-year university level. It is an excellent companion to Tayler's other two books in the series, *The Stars: Their Structure and Evolution* and *The Origin of the Chemical Elements.*

Galaxies are getting more and more popular in astronomy possibly because the number of known galaxies is increasing considerably as telescope instrumentation becomes more sophisticated. I never cease to be amazed at the statistic that there are about as many galaxies in the Universe as there are stars in our own Galaxy. Tayler doesn't cut corners in his book. Galactic properties are discussed with physical and mathematical precision. Considerable emphasis is given to the one galaxy that has been observed well, the one we are in, and there are chapters on observational results, stellar dynamics and the interstellar medium. Careful consideration is also given to the problems of estimating galactic mass and size and assessing the effects of chemical evolution. Other chapters deal with the different classes of galaxies and the origin and evolution of galaxies and the Universe.

This book will make galactic astronomy more accessible to many students and Professor Tayler is to be well congratulated for this.

Finally in the advanced section we have *The Astrolabe* by H.N. Saunders, a plastic folder which contains a 12-page booklet and an assembled, 14.5-cm diameter, white and clear plastic astrolabe. The astrolabe was invented in Alexandria around 400 AD and was used by astronomers for over 1,200 years. It gives the times of rising and setting of the Sun and certain specific stars throughout the year and their positions in the sky (that is, altitude and azimuth) at any time of the day and night. The astrolabe in the Saunders package has been designed for use at latitude 50° North but will still give useful answers within two degrees or so of this. If you want to know how astrolabes work and wish to become acquainted with such terms as retes, rules, plates, tympans, almucantars, heliacal risings and alidades this is just for you. There are 32 stars on the plate and the booklet is clearly written and contains many worked examples of astrolabe uses. The Saunders instrument is well made, durable and will stand up to a considerable amount of student handling. It would provide the basis of a very reasonable laboratory experiment for the more historically minded. A brief comparison can be made with another astrolabe package. In 1974 Roderick S. Webster produced an 8¾″ diameter, six section astrolabe kit, printed on gold cardboard and die cut so that the sections press out and don't have to be cut out (in 1974 it was $18 and obtainable from Paul MacAlister and Associates, Box 157, Lake Bluff, Illinois 60044). Six plates were included, for latitudes 30, 36, 42, 49, 52 and 56° North. It was decorated to conform with Renaissance practice. Webster's astrolabe looks more like 'museum' examples but is much more fragile than the smaller plastic Saunders one. Webster's booklet did stress the historical aspect more and had an excellent bibliography.

Let us now turn to the experimental aspects of astronomy. Like many other sciences, astronomy is both practical and theoretical and the majority of students studying the subject spend a considerable

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Particle physics

Norman Dombey

Elementary Particle Physics. By D.C. Cheng and G.K. O'Neill. Pp. 423. (Addison-Wesley: Reading, Massachusetts, 1980.) $29.50.

THIS is a very nice brief introductory text to the subject with roughly half devoted to electromagnetic and weak interactions and half to strong interactions. It is up to date: not only does charm enter together with strangeness in Chapter 1 but even the SLAC experiment of summer 1978 showing parity violation in electron scattering is quoted.

Nevertheless, the book is solid rather than fashionable and in spirit belongs to the late 1960s and early 1970s (this is not meant as a criticism) rather than the present. C,P and T-invariance; SU(3), (4) and (6); form-factors and structure functions; quarks and partons; dispersion relations and Regge Poles are all treated in some depth. Gauge theories are mentioned but not explained; so is the unification of weak and electromagnetic interactions; but QCD perhaps wisely is absent.

I will certainly recommend the book to my students, both undergraduate and graduate. There are, however, several errors in the text of the edition I received which need correcting. ☐

Norman Dombey is Reader in Physics at the University of Sussex, Brighton, UK.

A shining arc of the Sun's chromosphere (taken from *The Illustrated Encyclopaedia of Astronomy and Space;* Macmillan: London)

time doing observational and laboratory assignments. At my university the first-year students have to do 15 over a period of two terms. When it comes to preparing the instruction sheets for these projects I dip deeply into a whole range of books and manuals and when new books on astronomical experiments come onto the market I must admit that I buy them indiscriminately. Each book invariably contains a few good ideas usually distilled from the painful experience of the writer who has a similar job of organising laboratory and observing work for a large class of students. The British student doesn't usually expect to have to buy a laboratory manual. This is often not the case in America.

Projects and Demonstrations in Astronomy by D. Tattersfield is written for the overcast astronomer, and the author offers a range of projects that can be done indoors using only the most modest equipment and a modicum of mathematical skill. Many of these projects would be suitable for advanced level (about seventeen years old) students. In reading the book I came across many good ideas but too few projects which I would adopt in their entirety. I thought that too much use had been made of line drawings of photographic plates and spectra. This gives the student too little impression of the problems of measuring spectral lines on real plates. Often in astronomical spectroscopy the apparent width of the line varies as one moves along it. Also when it comes to measuring the distances between star images on plates the images are not points but diffuse and sometimes irregular disks. Reproductions of the original photographs can be produced fairly easily, as has been shown in the excellent series of laboratory exercises published by Sky Publishing Corporation from their journal *Sky and Telescope.* Tattersfield does this for the North American Nebula and I think should have done it more often.

More emphasis should also have been given to the errors in the quantities obtained from the graphical and numerical calculations. A student acquainted with the mathematics required to do these exercises should also know about standard deviation and the problems of fitting linear functions to small sets of data. In this context I am very worried when students are encouraged to plot the logarithm of the mass against the absolute magnitude for Sirius A and the Sun and then told "Draw a straight line through the two points on the graph. This is the mass–luminosity relationship." The author in the extensive "Solutions" section at the end of the book then adds data points for Capella A and Krüger 60A and inserts a much more reasonable line. I think I will use Tattersfield's book quite a bit, but only as a jumping off point, and with caution.

Astronomy: Activities and Experiments by Linda J. Kelsey, Darrel B. Hoff and John S. Neff is a revised and updated edition of the original 1971 manual which was published by the University of Iowa. After reading this book, two points become very clear. Firstly that the authors have had years of experience in the job of making laboratory exercises interesting and exciting for first-year university students and in dealing with the general range of student problems. Secondly they are first rate experimental scientists who know well the joys and frustrations of experimentation and the disciplines that must be developed when it comes to analysing and presenting the results. They end the brief section of suggestions for writing laboratory reports with the curt statement "Think before you write". Throughout the book it is obvious that the authors did just that. The book contains forty exercises, fairly evenly split between telescopes and observational techniques, coordinate systems and time, Earth, Moon and planets and the Sun, stars and galaxies, It is clearly presented, and well illustrated with a host of photographic reproductions. Each experimental text is subdivided into sections labelled purpose, references, introduction, procedure and discussion questions. It is an excellent book and I recommend it very strongly. It has been specifically designed for a two semester astronomy course at American universities but will be of inestimable use to all university, college and upper school teachers.

The book has spawned a baby brother. *Activities in Astronomy* by D.B. Hoff, L.J. Kelsey and J.S. Neff is aimed at the one-semester introductory astronomy student and is a shortened and somewhat simplified version of the previous book, containing only 25 exercises. The standard of presentation is just as good.

Now to the 'simple' books, by which I mean the ones designed for the non-mathematical American arts students market. I have been looking at three this year and these are Jay M. Pasachoff's *Astronomy: From the Earth to the Universe, Exploring the Cosmos* by L. Berman and J.C. Evans, and *New Horizons in Astronomy* by J.C. Brandt and S.P. Maran. *Horizons* and *Cosmos* are both second editions and Pasachoff's book is a slightly more historical version of his *Astronomy Now* which was a shorter version of his *Contemporary Astronomy* which was a shorter and no algebra and trigonometry version of his *University Astronomy . . .*

Where do we go from here? All the books cover the same areas of astronomy and are profusely illustrated with photographs of planets, stars, galaxies, clusters, and so on. If I were a student I would leave the choice of book to my course tutor. He has probably designed his teaching and his approach to astronomy around a specific text. The tests and examinations that must be passed will be based on that specific text. The scholar buys the book, learns the work, passes the exam, gets older and forgets astronomy. But look at it from a different angle. How should astronomy be taught to non-mathematical students? These three books illustrate two basic approaches. One is to treat astronomy as a simple story, which can begin historically, or at the edges of the Universe or at the Earth's surface or with our nearest star the Sun, and to tell the story with the aid of pictures. Pasachoff does this and so do Brandt and Maran. Both books do it well and colourfully, but I found myself time and again asking the question "Is it science?". The second approach is to treat astronomy not as a story, but as a science, a great science, the first science. The author can vary the difficulty of his approach but he never forgets that he is dealing with a science subject, a subject of experiment and hypothesis, of fruitful paths and blind alleys, a subject in which the mysteries of the unknown increase as our knowledge grows. Berman and Evans treat astronomy as a science. If I was teaching American arts majors about astronomy and trying to tell them what it is really all about I would use a book like Berman and Evans' *Exploring the Cosmos.* I would buy this book for some of the line drawings alone. It contains very little mathematics. That which is there can be understood by the most arty of art students given a bit of motivation. J. Robert Oppenheimer said "I believe most strongly in the nobility of this great effort to understand nature". Berman and Evans stress the how of astronomy as opposed to the other authors who spend too long simply describing the what.

Valerie Illingworth has edited *The Macmillan Dictionary of Astronomy* from contributions commissioned from a host of British professional astronomers. Dictionaries can be easily judged by asking two questions. The first is "Is it in?". Ignorance forces you to open the dictionary and the dictionary is only useful if it has an entry on the subject you are looking up. Secondly, "Does this entry tell you what you want to know?". The answer to the first question was "yes". The Macmillan dictionary nearly always had it in. Butler matrix, Bw stars, ylem, YY Orioris stars were all there. I only caught it out by straying into planetary geological features (grabens, horsts, and so on) and I should not have been looking these up in an astronomy dictionary anyway. I answered the second question in the affirmative too. So the conclusion is simple, if you want a good astronomy dictionary get this one. ☐

David W. Hughes is Lecturer in Astronomy and Physics at the University of Sheffield, UK.

Aerospace environment

T.B. Jones

The Upper Atmosphere and Solar-Terrestrial Relations. By J.K. Hargreaves. Pp.298. (Van Nostrand, Reinhold: Wokingham, UK, 1979.) £7.25.

THE launch of the first satellites in the late 1950s stimulated the study of space physics which has expanded rapidly since that time. The Earth's outer environment, or 'near space', is a particularly important and interesting region from both scientific and engineering viewpoints. It is the region where strong interactions with solar radiations and particles occur and where ionised particles can greatly influence man's activities.

Dr Hargreaves' book provides an excellent introduction to the aerospace environment. It is intended primarily for undergraduate use and in this respect it fills an important need, as there are relatively few books available on the subject at this level. The present volume provides a broad picture of the Earth's environment, beginning at the base of the ionosphere and extending outwards to the magnetospheric boundary. The structure and behaviour of the ionosphere and magnetosphere are discussed in detail and these topics form the major part of the book. The interaction of the Sun's emissions with the Earth's environment is of fundamental importance in understanding the behaviour of both the ionosphere and the magnetosphere and full coverage of this subject is included. In particular, a detailed account is given of solar disturbance phenomena and their spectacular consequences in the aerospace environment. Mathematical formulation has been kept to a minimum and is only introduced where essential to the understanding of a particular section. Chapters have been included on experimental techniques and on the engineering significance of the various regions described.

The material is well illustrated throughout and many cross-references have been included. In several cases numerical estimates of the magnitude of various parameters (for example, electric currents and fields) are included which help the reader relate them to his everyday laboratory experience. Copious references to more advanced literature are included at the end of each chapter.

The author has succeeded in presenting an integrated account of the wide range of phenomena which occur in the Earth's outer environment. He has done this in a clear and concise style which makes the book particularly attractive for undergraduate use. The work will also appeal to the more specialised reader who will find it helpful to have the wide range of topics, together with many references to source material, contained in one compact volume. ☐

T.B. Jones is Reader in Physics at the University of Leicester, UK.

Solar energy

D.O. Hall

The Sun. By D.K. McDaniels. Pp.271. (Wiley: New York and Chichester, UK, 1979.) Paperback £6.50.

THIS is a good book and certainly can be recommended as an introductory text for a course on solar energy, but . . . Why is it that so many books, articles, and so forth, on solar energy ignore the most important use of solar energy today, that is, the energy from biomass which daily provides the equivalent of 20 million barrels of oil (equal to USA oil consumption) — probably because such solar energy use mostly occurs in developing countries where it is of crucial importance. But it is not recognised that currently, for example,the USA obtains about 2% and Sweden about 10% of their total energy from biomass. Enough said!

The first three chapters discuss the overall energy problem, although it is heavily biased to the US situation. There are excellent chapters on the Sun and Universe and on solar radiation, and there are also discussions on economics.

The second half of the book describes solar energy applications for heating, cooling and electric power generation. The presentation of principles of operation, and the examples given, are very well thought out and clearly written with good diagrams and figures. The index and references are useful; the problems at the end of each chapter will also be helpful in teaching.

In short, a book to be recommended, as much experience in repeated teaching of a solar energy course to beginners is evident in the presentation. "The emphasis is entirely upon developing a qualitative approach of the subject" — this has been achieved. ☐

D.O. Hall is Professor of Biology of King's College, London, and Chairman of the UK Section of the International Solar Energy Society.

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Speech physics

C. J. Darwin

The Physics of Speech. By D. B. Fry. Pp.148. (Cambridge University Press: Cambridge and New York, 1979.) Hardback £9.50; paperback £3.50.

THE study of speech brings together an extraordinary variety of people. There are not just the phoneticians, old and new, transcribing and measuring, training their ears and improving their syntheses, but also a growing number satellites: computer scientists and electrical engineers intent on recognising and synthesising speech automatically, psychologists trying to understand how on earth we all manage to produce and perceive speech at all, speech pathologists helping those who can't and the theoretical linguists, phonologists and beyond, wondering what it all means.

Students in all these fields need to know the fundamentals of speech, but their teaching presents real problems as the engineer's meat is the pathologist's poison. Professor Fry's book is not for engineers. He manages, successfully, to explain the principles of the physics of speech assuming no mathematical ability in his reader (the only equation used is the definition of the decibel, and Fourier is not even mentioned). Nevertheless, all the necessary ideas are presented in a clear if not very sprightly way. Any student who has read the book carefully will understand the notion of a spectrum, appreciate the distinction between a sound source and its subsequent filtering, know what formants are and how to interpret a spectrogram, and will have some rudimentary knowledge of the acoustic cues to the more important phonetic distinctions. There are, of course, minor flaws, but nothing is seriously misleading.

The competition with which Fry's book has to contend is Ladefoged's *Elements of Acoustic Phonetics,* first published in 1962 and still available in paperback from University of Chicago Press for around two dollars. Ladefoged deals with wave analysis at greater length than Fry, illustrating his chapter with many clear diagrams of waves and spectra. Fry, with some justification, neglects the waveform for the spectrum. I suspect that students will find Ladefoged's treatment of this topic more lively than Fry's, and will appreciate his use of fewer words and more diagrams. But Ladefoged spends appreciably less time on actual speech sounds and their underlying production mechanisms, so that, faced with a real spectrogram, his reader would be considerably more baffled than Fry's. □

C. J. Darwin is Reader in Experimental Psychology at the University of Sussex, Brighton, UK.

Variegated manifestations

D. Rosen

Aspects of Biophysics. By W. Hughes. Pp.362. (Wiley: New York and Chichester, UK, 1979.) £11.95. *Biophysical Science.* By E. Ackerman, L.B.M. Ellis and L.E. Williams. Second edition. Pp.634. (Prentice-Hall: Englewood Cliffs, New Jersey, and Hemel Hempstead, UK, 1979.) £18.20.

HUGHES quotes a US National Academy of Sciences study which argues that "the classical subdisciplines of biology [that is, zoology, botany, and so on] are insufficiently instructive as approaches to current understanding and appreciation of life in its varigated [*sic*] manifestations". He uses this as a valid justification for cutting through the range of biological phenomena in an orderly way, with the order determined by those physical phenomena which can be discerned among the biological ones. Ackerman and his co-authors find the subject organised in almost the same sequence as that found by Hughes, but they tackle it in the reverse order. The two books are at just about the same level, textbooks suitable for senior undergraduates or junior graduate students.

The problem of teaching biophysics now is akin to the problem of teaching biochemistry about 50 years ago. Then, the question was whether any students would have the requisite background in chemistry and biology: they were likely to have one but not both. Now, the question is whether they have the background of physics, chemistry and biology: they may well have the first two or the last two, but not all three. The two books considered here both affect, in the enthusiasm of their prefaces, to be appropriate to all classes of readers. In a rough way, however, it may be said that Hughes' book is more suited to the student with the physics-chemistry background and Ackerman's to the student with the biology-chemistry background.

Hughes starts with atoms and molecules and with X-ray diffraction and electron microscopy as the techniques of studying the most elementary parts of living things. He builds upwards. He goes on to describe the physics of proteins and nucleic acids, and then membranes and mitochondria, so leading to physical aspects of cellular function and organic function, and finally to still larger problems of living matter. What I particularly liked in his approach is the way he pushes a numerate, mathematical description into quite elementary accounts of the various topics he deals with, from enzyme action to vision. When the topic itself has a well established mathematical formulation, he takes full advantage of it. In his account of nerve action potentials, for example, he gives brief but adequate derivations of cable theory and the Goldman equation for transmembrane potential before going on to obtain the Hodgkin–Huxley equation. However, this is strictly the physics or physical chemistry of the matter — it comes with only the barest introduction and practically no account of the biological consequences. What I found most distressing in Hughes' book were the misprints and spelling errors, starting on line 3 of page ix, as quoted above, and continuing liberally through the text, striking alike at formula and English word.

Ackerman, Ellis and Williams, in the second edition of a book originally by Ackerman alone, have kept to the plan of the first edition. This combines a partly historical approach with some pedagogical insight into what might tempt a student to give attention to topics in biophysics. The Preface proposes Helmholtz as the originator of biophysics and the first chapters deal with Helmholtz' major interests, hearing and vision. The development is then, as it were, analytic in contrast to Hughes' synthetic approach — the electrically sensitive tissues of the body are considered, leading on to other interaction of cells with physical stimuli, to the physical features of cellular function, to the constituent parts of cells, to biological macromolecules, and to the thermodynamics of biological function. In further contrast to Hughes, Ackerman and his colleagues seem to pull in mathematical explanation with some reluctance when it can hardly be avoided, but prefer a descriptive approach. In their account of nerve action, they give a brief explanation of the Hodgkin–Huxley equation but do not mention cable theory or even the Nernst or Goldman formulae for transmembrane potential. In most other cases, however, they end up by giving much the same physics in much the same mathematical form as is given by Hughes, but accompany it with fuller verbal explanation and better indications of the implications for the study of biology. Ackerman and his co-authors devote the final part of their book, a sixth of the whole text, to biophysical techniques and instrumentation and to medical applications of biophysics. That this section, seventeen years later, is the only one which required major recasting from the first edition, shows how well Ackerman judged his original text; and the second edition preserves the confidence and balance of the first.

From the comments made here it can

perhaps be inferred that these two books, although coming to biophysics in opposite ways, are not complementary: they are just different and each has its virtues. Ackerman is nearly twice as long (and nearly twice as expensive) as Hughes, and so contains lengthier accounts, but nearly all topics found in one also appear in the other. However, though I can imagine few students with a biological background preferring Hughes to Ackerman, there are some students from physics who would prefer Ackerman's approach to that of Hughes. My own preference is marginally in favour of Hughes — I found his style the livelier of the two, his approach to topics more engaged, and the references he provides more interesting and research-oriented. Both books are to be recommended, however, as serious introductions to biophysics, and both are better than the two or three comparable texts which have recently appeared. □

D. Rosen is Head of the Department of Biophysics and Bioengineering at Chelsea College, University of London, UK.

Introductory immunology

George Feinberg

Immunology at a Glance. By J.H.L. Playfair. Pp.35. (Blackwell: Oxford, 1979.) £2.25. *Immunology Simplified.* By T.R. Bowry. (Oxford University Press: Oxford, 1979.) £2.50. *Textbook of Immunology.* By B. Benacerraf and E.R. Unanue. Pp.298. (Williams and Wilkins Company: Baltimore, 1979.) $10.95.

THE only thing these three books have in common is the word "immunology" in their titles. No three authors could have taken three more diversified approaches to their common subject. Yet, this is not to fault any of them, since immunology has become so complex an author must take a calculated decision on how he can best present it to the would-be learner.

J.H.L. Playfair's *Immunology at a Glance* chooses to do it essentially with diagrams (sketches, the author calls them): a set of 34, in fact, which starts with the "Scope of Immunology" and then proceeds stepwise in complement cascade fashion, each diagram 'activating' the next till the "terminal assembly" of three diagrams representing "Altered Immunity". The sequence is logical and each diagram is accompanied by a brief — I would say too brief — explanatory section, as well as a useful listing of definitions.

But the title is misleading. Immunology *at a glance* the book certainly is not. The diagrams are often complex: one picture may be worth a thousand words, but not when it tries to express a 'thousand' ideas. Even with the accompanying explanations, most of the diagrams require a study session rather than a glance. That said, it is a good book, based on a novel approach, but ill-served by its title, which suggests it is a casual book for the beginner. In fact, in his preface the author states "this is not a book for immunologists". I dispute this! I see its greatest potential as an *aide memoire* to the professional immunologist and, especially, to the teacher of immunology, who, more than anyone else, must try to grasp the whole picture. I suggest a more appropriate title for this book would be: "An Atlas of Immunology".

Alas, as in all first editions, some errors have crept in: such as a definition of lysosome which gives the impression the macrophage is its sole source; and the diagram on "Anaphylaxis and Allergy", which has bradykinin emerging as a mediator from the mast cell. I must also note my sorrow at one glaring omission: the failure to give due consideration to the structure and characteristics of antigens, without which there would be no immunology.

The book by T.R. Bowry is antipodean to that of Playfair. *Immunology Simplified* it certainly is; some may feel it is over-simplified. It reduces diagrams to a bare essential and relies, instead, on a narrative-style text to make the immunological feast palatable to the uninitiated. In this it largely succeeds, but — perhaps inevitably — at the sacrifice of any profundity. Without too much effort, it could be transformed into a book of popular immunology for the layman.

But perhaps therein lies its strength: it follows a logically flowing pattern, setting forth the fundamentals in three brief chapters, before ranging over the consequences — good and bad — of the activation of the adaptive immune system. Adding to the sense of simplicity — "extreme simplicity" is the publisher's assessment on the back cover — is the small-page format and the large, open print. This is a book which succeeds in living up to its title; and if it is 'simple' immunology the reader is seeking, he need go no further in his quest.

The last of the trio, Benacerraf and Unanue's *Textbook of Immunology*, comes closest to the traditional idea of a college textbook. With its informal offset lithography it gives the impression of being the original lecture notes of an immunology teacher. Perhaps this is not surprising, as it "was originally organised from the teaching materials . . . (used) as part of the introductory course in immunology of the Harvard Medical School curriculum". The best praise I can give it is that it certainly lives up to the standards one would expect from an institution of Harvard's repute.

Again, we have a type which is large and easy on the eye. This makes the mastering of the wealth of up-to-date information in the book a less formidable prospect. The development of the subject is classical and, to my way of thinking, logical in devoting a whole chapter to a consideration of antigen, before launching into the immune response it induces. Perhaps less logical is the presentation of the complement system long after a series of chapters in which a familiarity with its workings would be useful; but in a book that so elegantly sets out the precepts of immunology, this is mere quibbling. Particularly lucid presentations of complex subjects are the chapters on transplantation biology, immune regulation and immunogenetics. Diagrams are sparse, but clear and pertinent. I found no obvious errors of omission; nor, at a quick reading, any of commission. Pleasing, too, is the short list of both classical and current references appended to each chapter.

So here we have three books collectively catering for a wide variety of tastes and needs. It is for the reader to choose that most suited to his immunological appetite and/or dietary requirements. □

George Feinberg is Director of Studies in Immunology at Chelsea College, London, UK.

Participating in perception

F. W. Campbell

Sensation and Perception. By S. Coen, C. Porac and L. M. Ward. Pp.514. (Academic: New York, San Francisco and London, 1979) Hardback $14.95; £9.75; paperback £6.50.

THIS textbook has several unusual features which make it a particularly useful teaching tool. Firstly, it is extraordinarily well illustrated with numerous clearcut diagrams to distill succinctly the relevant information. To illustrate the properties of colour vision there are also a number of colour plates. One of its strongest features is the Demonstration Boxes, which contain instructions for the reader to undertake numerous simple experiments. For example, pouring out a glass of water reflected in a ladies' hand-mirror. To quote from another Box, "use two white plastic spoons (like those probably available in any campus cafeteria) to produce a Ganzfeld by placing the bowls of the two spoons over each eye as shown in the accompanying figure". Again, sit in your motor car, turn on the radio and start the engine. Change the engine speed and note the effects of the engine noise on the quality and perception of some classical music. This encouragement of the reader to participate does much to deepen their understanding of the text.

To cover the whole range of sensation and perception has required three authors. Yet the style is very uniform and well integrated. Indeed, another unusual feature is to intermingle the different sensations rather than segregating each one to its individual section.

For the newcomer there is a comprehensive glossary of specialised terms, words and units. There are 23 pages of references containing some 500 scientific papers to enable the reader to study in depth any particular point. There is also a good author and subject index.

Recent advances are often handled by inserting a Special Topic, such as, How to Calculate d-prime, or, What is the Neural Response? or, The Modulation Transfer Function. This enables the newcomer to skip these special points on his first reading.

The publishers are to be congratulated on producing such an attractive volume for a remarkably low price. ☐

F. W. Campbell is Reader in Neurosensory Physiology at the University of Cambridge, UK.

How the mind works

Sandy Lovie

Cognitive Psychology. By W.A. Wickelgren. Pp.436. (Prentice-Hall: Englewood Cliffs, New Jersey and Hemel Hempstead, UK, 1979.) £11.

THIS is the latest offering from the ever flowing pen of one of the leading lights in human information processing and cognition. In a (relatively) brief compass Wickelgren deftly sketches in most of the live areas and issues of this loosely articulated and most exciting area of psychology. From such traditional areas as perception (both visual and auditory), imagery and attention, thinking, reading and long- and short-term memory, to the more novel topics of semantic memory, Wickelgren has assembled a most persuasive body of evidence to support his modest contention that "Cognitive psychology is concerned with the general principles of how the mind works". Moreover Wickelgren's ability to synthesise material and to disambiguate the closely argued efforts of most of today's brightest workers gives the reader the exciting and restless feeling of being the edge of current knowledge (fe references are earlier than 1960, most ar from the middle to late 1970s).

Of course, Wickelgren cannot hope t avoid the larger questions hanging over th subject: for example, is it structure *an* process or structure *or* process; is it a happening in here or out there; what abo the issues of application and ecologic validity; how far can we push the comput analogy? Perhaps it is inappropriate to as so committed a member of the cognitic club to bother too much about these issues as their lengthy (and usually ponderous) consideration tends to spoil the fun in describing the experimental infighting. But these problems are neither trivial nor vacuous simply because of their low rating on the excitement scale.

Three final points: the chapter objectives and summaries are useful both for insightful and memorial purposes; thank God for the lack of references to Thomas Kuhn, paradigms, normal science, and so on; please when can we have a paperback version for our impoverished students? ☐

Sandy Lovie is Lecturer in Psychology at th University of Liverpool, UK (Acting Hea 1979-80).

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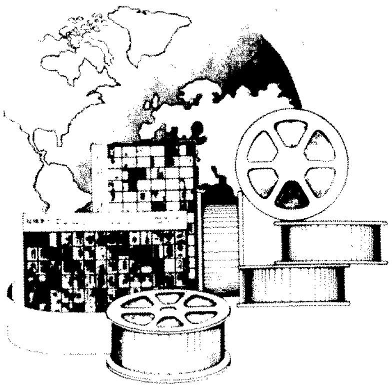

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Behaviour of experimental animals

B.J. Sahakian

Animal Behaviour in the Laboratory. By P. Silverman. Pp.409. (Chapman and Hall: Andover, UK, 1979.) £15; paperback £8.50.

THE author has attempted to write a simple, informative, and interesting account of the methods available for the observation and quantification of the behaviour of experimental animals. The dust-jacket indicates that the text is suitable for use by "second and third year undergraduates studying animal behaviour and psychology; to postgraduates and to industrial research workers in pharmacology and toxicology laboratories". Its 25 chapters cover a range of tests currently used by researchers interested in determining the effects of drugs on behaviour. The tests include those for the investigation of both conditioned and unconditioned behaviours, and the emphasis is on the behaviour of rodents. The chapters are varied and discuss such topics as ethics, experimental design, physiological psychology, drug screening, central nervous system pharmacology, motor activity, exploratory behaviour, self-administration of drugs and brain stimulation, classical and operant conditioning, and social behaviour.

Although there are some excellent texts for the expert or the advanced student in this area, this book could possibly fill a need for the beginning student or the worker in industrial pharmacological or toxicological research wanting to acquire behavioural skills. Unfortunately, however, though the author has chosen his topics well, their treatment is disappointing. The style of writing is sometimes vague and often lapses into homespun philosophy, where opinions are not substantiated by reference to published research. This tendency is irritating when such important issues as the widespread abuse of barbiturates as sleeping-pills in the UK is discussed for example, page 37. In general, the text could be supplemented by more references, particularly in certain chapters (e.g. chapter 11) to work done after 1970.

Balancing these faults, the summaries at the ends of most chapters are clear and concise, and the different indices (General, Chemicals, Species, and Author) are a useful feature. Finally, it is obvious that the author has a sincere concern for ethical standards in procedures used with experimental animals, a matter of considerable relevance for a general audience. ☐

B.J. Sahakian is a Research Scientist at the MRC Dunn Nutrition Unit, University of Cambridge, UK.

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SRC CASE STUDENTSHIP

'Control of neurotransmitter release'

Applications are invited from graduates with a good honours degree for an SRC CASE Studentship to be held in the Department of Pharmacology in conjunction with ORGANON LTD. from September 1980. The project will be supervised by DR I E Hughes and will involve an investigation of the mechanisms controlling release of radiolabelled neurotransmitters from superfused brain slices. Organon may supplement the normal SRC studentship and will pay additional expenses when the student works in Organon Research Laboratories in Oss (Holland) and Newhouse (Scotland).

Applications, including curriculum vitae, should be sent to Dr I E Hughes, Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9JT (Tel. (0532) 31751 Ext 7435) by 30 March 1980. 4514(F)

UNIVERSITY OF ABERDEEN

Faculty of Medicine

RESEARCH STUDENTSHIPS

Applications are invited from recent first or upper second class honours graduates in a biological science subject, and from those who expect so to graduate in 1980, for research studentships established by the Faculty of Medicine from its Medical Endowment Funds. Successful applicants will study for the degree of PhD on an approved project in one of the Departments of Anatomy, Biochemistry, Obstetrics and Gynaecology, Pathology, Pharmacology, Physiology or Surgery, commencing in October 1980.

The value of the studentship is £1,820 pa (for students living away from home) with payment of tuition fees and other allowances.

Application forms and further particulars of the projects may be obtained from Helen E Cunningham, University Office, Regent Walk, Aberdeen AB9 1FX to whom applications should be submitted by 21 March 1980. 4527(F)

COURSE

UNIVERSITY OF LONDON
Royal Postgraduate Medical School

MSC DEGREE COURSE IN EXPERIMENTAL PATHOLOGY (TOXICOLOGY)

Applications are invited for the above, one-year, full-time course which is being offered from 1 October 1980.

Applicants should possess a first degree in medicine, veterinary medicine or appropriate science subjects.

A limited number of MRC course studentships will probably be available.

Further details and application forms are available from: School Office (MSc), Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS. Telephone: 01 743 2030 ext 351. Closing date for applications: Friday 30 May 1980. 4530(D)

WORKSHOP

CSH

COLD SPRING HARBOR LABORATORY

WORKSHOP ON

FORMS OF MICROTUBULAR ORGANIZATION IN CELLS

MAY 14 – MAY 17, 1980

G. Albrecht-Buehler, Cold Spring Harbor Laboratory
R. G. W. Anderson, The University of Texas

The workshop will focus on biochemical, morphological and functional aspects of microtubules in eukaryotes through presentations by invited speakers and follow up discussions and poster sessions.

Abstracts are invited to be submitted for the poster sessions by March 28.

Further information, registration and abstract forms can be obtained by contacting:

Mrs. Gladys Kist, Meetings Secretary
Cold Spring Harbor Laboratory
P. O. Box 100
Cold Spring Harbor NY 11724

W818(V)

COURSE

EMBO COURSE ON MEMBRANE BIOGENESIS

October 5-15, 1980
European Molecular Biology Laboratory
Heidelberg, F.R.G.

The course will be organized by the Membrane Group at EMBL and will comprise a series of lectures (6-8 October) and practical work (9-15 October).

The teaching staff will include:

G. Blobel (USA), N. Crawford (UK), N. Le Douarin (France), C. de Duve (USA), C. G. Gahmberg (Finland), G. Gerisch (F.R.G.), V. Herzog (F.R.G.), E. Lazarides (USA), J. Meldolesi (Italy), W. Neupert (F.R.G.), B. Pearse (UK), D. Sabatini (USA), R. Schekman (USA), J. Slot (Netherlands), M. Schwartz (France) and R. Steinman (USA).

and the following staff members of EMBL:

B. Dobberstein, H. Garoff, G. Griffiths, A. Helenius, B. M. Jockusch, D. Louvard, H. Reggio, K. Simons, G. Warren, H. Weiss and P. Wingfield.

Practical work will include: Membrane assembly in vivo and in vitro; Intracellular transport of membrane proteins; Biogenesis of cell polarity; Endocytosis and membrane recycling; Immunofluorescent microscopy and electron microscopy of the cytoskeleton.

A maximum of 36 students will be allowed to attend. Students will be allowed to pursue one of the courses above, but in their application they should also indicate which of the other topics is an acceptable alternative. Applications should include a short curriculum vitae and a description of present research interests.

Applications should be sent to: Henrik Garoff, Postfach 10.2209, D-6900 Heidelberg, F.R.G.

The closing date for applications is May 31st, 1980.

W821(D)

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